ABSTRACT

HATHAWAY, JON MICHAEL. An Evaluation of Indicator Bacteria Transport in Stormwater Runoff and Removal in Stormwater Control Measures. (Under the direction of Dr. William F. Hunt III.)

Microbial quality in surface waters is a concern across the United States, Europe, Australia, and elsewhere due to human reliance on surface waters for food, recreation, and other life sustaining activities. Although pathogens are of utmost concern, indicator bacteria are typically used for regulatory purposes to indicate the presence of fecal matter, and thus the possible existence of pathogens. Total Maximum Daily Loads are established for surface waters impacted by excessive indicator bacteria. Analyses are required to categorize sources of indicator bacteria, and a plan is developed to restore water quality in the impacted water by way of various management/control practices. Stormwater runoff has been shown to have high indicator bacteria concentrations, contributing to microbial degradation in surface waters.

Although numerous studies have been performed to establish patterns of indicator bacteria transport and export in estuarine and riverine systems, relatively little research has been performed for urban stormwater (prior to runoff entering surface water). Chapter 2 provides an analysis of variables which may influence indicator bacteria export from an urban watershed. Event Mean Concentrations (EMCs) of *E. coli* and fecal coliform exhibited significant seasonal variation ($p < 0.05$). Based on multiple linear regression analyses, EMCs were also influenced by antecedent meteorological conditions, with temperature and moisture being important in explaining variability among sampling events. Further analysis in Chapter 3 provided a traditional first flush assessment of data collected from the urban watershed. Although total suspended solids (TSS) exhibited a first flush in the watershed, no first flush effect was noted for *E. coli* and enterococci, and the first flush effect for fecal coliform was relatively weak. Seasonal variations in first flush strength were

observed, likely due to differences in pollutant sources between seasons. These studies emphasized the importance of seasonality and antecedent conditions in indicator bacteria transport and export from urban watersheds. Further, the lack of a substantial first flush effect suggests stormwater control measures ("SCMs," also known as Best Management practices or "BMPs") cannot sequester proportionally more indicator bacteria as a result of greater mass delivery during the beginning of storm events.

Stormwater runoff is typically managed by implementation of SCMs. Although SCMs have been shown to sequester numerous pollutants, relatively little is known regarding their ability to sequester indicator bacteria. The effectiveness of SCMs in Charlotte, NC, and Wilmington, NC, was examined in Chapters 4 and 5, respectively. Differences in performance were noted between the two locations, potentially due to differences in particle association of indicator bacteria between the relatively clayey soils in Charlotte, NC, and the sandy soils in Wilmington, NC. High water tables in Wilmington, NC, likely also influenced results, particularly for wet ponds. Although some SCMs showed statistically significant reductions of indicator bacteria ($p < 0.05$), some SCMs appeared to export indicator bacteria. Further, effluent indicator bacteria concentrations were observed to vary seasonally. Well performing filtration/infiltration-based SCMs were examined in both locations; however, a paired watershed study in Wilmington, NC, showed differing performance between two bioretention cells. These differences were explored in Chapter 6, and soil media depth was identified as the most likely difference between the two cells leading to differences in indicator bacteria sequestration. These data suggest SCMs do possess treatment mechanisms which are effective at sequestering indicator bacteria; however, an environment may be present in some SCMs which allows indicator bacteria to persist and/or regrow. Infiltration-based SCMs offer some advantage, as mass removal of indicator bacteria can be realized through infiltration of runoff into

subsoils. However, the impact of these practices on groundwater microbial quality should be investigated. For bioretention cells, a minimum soil media depth appears to exist, below which poor sequestration of indicator bacteria may occur due to high soil water flux and low contact time. Finally, seasonal variations in effluent indicator bacteria concentrations from SCMs should be considered in TMDLs.

An Evaluation of Indicator Bacteria Transport in Stormwater Runoff and Removal in Stormwater Control Measures

by
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DEDICATION

To Earth and Sky and Water

What man could grasp your complexity?

You call to that which is vast and deeper still

Oh, if I could but live with such purpose

BIOGRAPHY

Jon Michael Hathaway was born on June 7, 1980 in Norwich, Connecticut. He is the son of Richard and Deborah Hathaway of Charlotte, North Carolina, and the youngest of three boys. Jon attributes his interest in the environment to hours spent along the rocky Connecticut shore, cross country skiing in the mountains, and various other outdoor adventures undertaken as a child.

Jon's family settled in North Carolina in the summer of 1996. In the spring of 1998, Jon graduated from high school and enrolled at North Carolina State University. He majored in Environmental Engineering and received his bachelor's degree in December 2002. Upon graduation, Jon continued his studies in the Department of Biological and Agricultural Engineering at North Carolina State University under the direction of Dr. Robert Evans. Jon studied constructed wetlands as a practice for remediating groundwater contaminated by swine lagoon seepage. Jon completed his master's degree in May 2005 and took a position with North Carolina State University as an extension associate working for Dr. William F. Hunt.

It was during his time working as an extension associate that Jon's love of teaching and research became apparent. Jon took part in 25 cooperative extension training events and was involved with the design, construction, and/or monitoring of over 20 stormwater best management practices. Jon decided academia was a great fit for his interests and abilities, and returned to school to pursue his doctorate in January 2008. Jon studied urban stormwater management under the direction of Dr. William F. Hunt, with particular focus on indicator bacteria in urban runoff.

Jon currently lives in Raleigh, NC, with his wife, Amy, and their two cats, Sirius and Isco.

ACKNOWLEDGEMENTS

There is a phrase used during microbiological analyses when a Petri dish is so full that microbe colonies can't be quantified. The number is simply recorded "Too numerous to count." While this can be unsatisfying and extremely difficult to build conclusions upon, it aptly describes my predicament in acknowledging those who have led me to this point. I will try, likely in vane, but all should recognize that one does not complete a Ph.D. without extensive support……and perhaps some luck as well (so happy there was no drought between mid 2008 and mid 2009).

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To my family, you instilled in me the desire to work hard, to lend a hand to those who need help, and to treat those around me with respect. Mom and Dad, you are an inspiration to me. I always felt encouraged that I was only constrained by my own desire to work and excel. Thank you for always finding a way to give me every opportunity you could. I also thank you for teaching me that a man's legacy extends far beyond his success in a career. Joel and Pete, you guys are two of my best friends. Thank you for always being there to listen to me, for giving advice, and for helping me wade through these last few years. To my friends and family at Visio Dei, I love all of you. I cannot imagine a more supportive and fun community to be a part of.

To Sirius and Isco, thank you for always getting me up on time….between 4:30 and 5:30 am every day. No wait…..I take that one back.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 Literature Review: An Evaluation of Indicator Bacteria Transport in Stormwater Runoff and Removal in Best Management Practices

1.1 Introduction - Regulations Leading to the Management of Stormwater

1.1.1 Clean Water Act

In 1948, the Federal Water Pollution Control Act was passed in an effort to reduce water pollution in the United States. Amendments were made in 1972 and 1977, and the legislation became known as the Clean Water Act. The intent of the Clean Water Act was to address water quality within the United States, setting goals for providing fishable and swimmable waters and prohibiting the discharge of toxic substances (Viessman and Hammer 1998). Originally, the Clean Water Act regulated the discharge of pollutants from point sources such as industrial and domestic wastewaters, making it illegal to discharge from such point sources without a permit. The USEPA's Pollutant Discharge Elimination Program (NPDES) permit program was implemented to facilitate this portion of the Act.

By 1987, non-point source pollution had been identified as a major source of pollution in surface waters in the United States. The Act was further amended to require phased NPDES permits for stormwater discharges within the United States. These NPDES permit requirements included stormwater generated during some industrial activities and from municipal separate storm sewer systems, called MS4s. The NPDES requirements for municipalities were staged into two Phases. Phase 1 included municipalities with a population larger than 100,000 and was established in 1990. Phase 2 included municipalities with a population less than 100,000 and was established in 1999. To comply with these NPDES regulations, municipalities must establish a program which includes:

- Public education and outreach in stormwater impacts
- Public involvement / participation
- Illicit discharge detection and elimination

- Construction site stormwater runoff control
- Post-construction stormwater management in new development and redevelopment
- Pollution prevention / good housekeeping for municipal operations

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1.1.2 Clean Water Act Section 303d

Section 303d of the Clean Water Act served to further define and take action against surface water degradation in the United States. Surface waters which exceed water quality standards for a given pollutant, such that they cannot be used as intended, become part of the 303(d) list of impaired waters. In an effort to restore surface waters on the 303(d) list, management plans are developed which establish the total maximum daily loading (TMDL) of a pollutant of concern that can be discharged into a given surface water while still maintaining its designated use. These TMDLs address both point and non-point sources and help establish a course of action for watershed managers. If non-point sources are determined to be a major contributor to degradation of a given surface water, states can apply for EPA funded section 319 grants to help fund restoration activities.

1.1.3 Use of Stormwater BMPs in Achieving Water Quality Goals

To comply with the regulatory demands associated with Phase 1 and Phase 2, and to achieve TMDLs for surface waters listed on the 303(d) list, non-point source stormwater must be treated. Stormwater Best Management Practices ("BMPs," also known as Stormwater Control Measures or "SCMs") are commonly installed in newly developed urban watersheds or retrofit into existing urbanized areas to mitigate the impact of non-point source stormwater runoff. BMPs are an integral part of watershed restoration activities and often provide the only treatment of stormwater runoff prior to discharge into surface waters. Structural stormwater BMPs include wet ponds, dry detention basins, wetlands, bioretention areas (which function as filtration systems), and proprietary devices. Proprietary, or manufactured, devices use baffles, swirl flow patterns, settling chambers, and other means to separate floatable and settleable solids from stormwater runoff. Each BMP provides some combination of natural treatment mechanisms, and BMPs have been shown effective at sequestering and removing a range of pollutants (USEPA 1999). However, as pollutants of concern continue to emerge in urban landscapes, and TMDLs are established for these

emerging pollutants, current BMP design standards must be tested to determine if they are effective for these pollutants.

1.2 Pathogenic Microorganisms

Microorganisms are common in the natural environment, performing beneficial functions such as nutrient cycling, decomposing organic matter, and enhancing plant productivity through symbiotic relationships (Sylvia et al. 2005). The term *microorganism* refers to many different organisms including bacteria, protozoa, and fungi. Although often beneficial, some types of microorganisms can cause sickness when they enter the human body during consumption of contaminated shellfish, ingestion during water-related recreational activities, and even through skin contact with contaminated waters (USEPA 2001). Microorganisms (and viruses) that cause illness are referred to as *pathogens* and are a major concern when they are present in streams, lakes, and marine waters. Pathogens can be bacteria, protozoa, or viruses. Common examples of pathogens are presented in Table 1.1.

Table 1.1: **Pathogen types, descriptions, and examples**

Type	Brief Description	Example Pathogens (disease)
Bacteria	Single-celled organism with no nuclear membrane. Cell structure is simple, containing few organelles.	*Salmonella* (Salmonellosis), *Escherichia coli 0157:H7* (Gastroenteritis), *Vibrio cholera* (Cholera), *Salmonella typhi* (Typhoid fever)
Protozoa	Single-celled organism, genetic material enclosed in nuclear membrane. Described as microfauna. Often feed on bacteria, algae, and other microorganisms.	*Giardia lamblia* (Giardiasis), *Cryptosporidium* (Cryptosporidiosis), *Entamoeba histolytica* (amoebic dysentery)
Virus	Infectious agent consisting (structurally) of either DNA or RNA covered in a protein coat.	Hepatitis A (infectious hepatitis), Rotavirus (Gastroenteritis), Adenovirus (respiratory disease, gastroenteritis)

1.3 Indicator Bacteria

Indicator species are used to test for the possible presence of pathogens in surface waters. While these species may not be harmful to humans themselves, their presence in surface waters indicates contamination from warm-blooded animal fecal matter. Fecal matter can contain harmful viruses,

bacteria, and protozoa (Myers et al. 2007). Various indicator bacteria have been used to assess water quality degradation due to pathogens including: total coliform, fecal coliform, *Escherichia coli* (*E. coli)*, and enterococci. In 1986, the USEPA's Ambient Water Quality Criteria for Bacteria report (USEPA 1986) discussed the merits of various indicator bacteria and recommended either *E. coli* or enterococci for use in freshwater environments and enterococci as an indicator in marine environments. The criteria stated that for fresh waters designated for use as full body contact recreational waters, the geometric mean over a 30-day period should not exceed 126 col/100 ml for *E. coli* and should not exceed 33 col/100 ml for enterococci. For similarly designated marine waters, the geometric mean over a 30-day period should not exceed 35 col/100 ml for enterococci.

A literature review by Wade et al. (2003) concluded that the USEPA bacteria standards set forth for marine waters were supported by the available literature. For fresh waters, Wade et al. (2003) indicated that *E. coli* were a more consistent predictor of gastrointestinal illness than any other indicator. Despite this, fecal coliform remains a commonly used bacteria indicator for surface waters. The recommendation for fecal coliform, set in 1976 by the USEPA, is the log mean over a 30-day period should not exceed 200 CFU/100ml (colony forming units per 100 ml) and no more than 10 percent of the samples should exceed 400 CFU/100ml (USEPA 1976). As of 2003, 18 states had adopted the *E. coli* standard for fresh water, 6 states adopted the enterococci standard for fresh waters, and 9 states had adopted the enterococci standard for marine waters (USEPA 2003a). Thus, as there are still a variety of indicator bacteria used for water quality standards, research performed on fecal coliform, *E. coli*, and/or enterococci is valuable in the ongoing effort to manage indicator bacteria through models and TMDL development.

1.4 Water Quality Degradation Due to Pathogens

In the United States Environmental Protection Agency's (USEPA 2008) National Water Quality Inventory in 2006, approximately 12% of the river and stream miles that were surveyed were impaired by indicator bacteria. Of the stream and river miles designated as impaired, either unable or partially unable to meet their designated use, more were impacted by this pollutant than by any other. Indicator bacteria were also the number one source of impairment in bays and estuaries, the number two source of impairment in oceans and near coastal areas, and the number three source of

impairment along coastal shorelines (USEPA 2008). In light of the negative impact that pathogens have on surface waters in the United States, TMDLs have been established for impaired water bodies. Municipalities across the country are exploring options to reduce indicator bacteria inputs from point and non-point sources.

1.5 Pathogen Sources and Health Impact

Pathogens and indicator bacteria have a number of sources in urban environments. Microbes originating from humans can enter surface waters from various point sources such as septic systems, sanitary sewer overflows, and leaks in sanitary sewer pipes (Graves et al. 2002, Booth et al. 2003, Line et al. 2008, Cahoon et al. 2006, Arnone and Walling 2007, USEPA 2001). Non-human sources are also prevalent in urban watersheds. Domestic animals, wild animals, and garbage are all sources of contamination (USEPA 2001, Young and Thackston 1999, Mallin et al. 2000, Weiskel 1996).

Numerous studies have shown that development in watersheds leads to increased export of indicator bacteria. In a study of 18 mixed land use watersheds in West Georgia, Schoonover and Lockacy (2006) noted that watersheds consisting of greater than 24% imperviousness exhibit higher fecal coliform concentrations than watersheds with impervious percentages less than 5% during both base and storm flow. Studies by Line et al. (2008) and Mallin et al. (2000) conclude similarly that urbanization in watersheds leads to increases in indicator bacteria export.

Pathogens originating in urbanized areas pose a public health risk. This is a concern in both freshwater and ocean environments where recreation and consumption of aquatic organisms can lead to exposure to harmful organisms (USEPA 2001). A substantial amount of research has examined the impact of bacterial pollution on ocean environments. In Santa Monica Bay, California, Haile et al. (1999) showed an increased risk of health effects to swimmers located closer to storm drain outlets, noting that higher levels of bacterial indicators were found near the storm drain. Likewise, a study by Curriero et al. (2001) evaluated 528 waterborne disease outbreaks reported in the USEPA disease database from 1948 to 1994 by comparing them to precipitation data from weather stations geographically near the location of the outbreak. Precipitation amounts were

summed monthly at the weather stations and ranked over the reporting period. Curriero et al. (2001) found a significant relationship between extreme precipitation events and disease outbreaks. Specifically, disease outbreaks which were linked to surface waters had a strong relationship with extreme precipitation events during the month of the outbreak. Extreme events were defined as months during which high amounts of precipitation fell relative to other months from 1948 to 1994.

1.6 Survival, Die-off, and Transport in Urban Watersheds

To explore the transport and removal of harmful microorganisms, an understanding of how they persist in natural environments is important. Various environmental conditions influence pathogen and indicator bacteria survival, die-off, and transport in urban environments. These conditions include temperature, moisture conditions, pH, predation, exposure to sunlight (UV radiation), and nutrient availability (USEPA 2001, Arnone and Walling 2007, Stevik et al. 2004, Ferguson et al. 2003). The degree to which these factors influence survival, die-off, and transport in urban environments is an ongoing area of research within the scientific community. Understanding these factors may lead to more accurate models and more effective management strategies.

Indicating that there is limited information regarding microorganism behavior in urban environments, McCarthy et al. (2007) examined *E. coli* Event Mean Concentrations (EMCs) and loads from four urban watersheds in Melbourne, Australia, and correlated them to various environmental factors. The factors were selected based on known processes leading to the survival, die-off, and transport of microorganisms in the environment and included water quality variables, rainfall characteristics, runoff characteristics, and meteorological factors ranging from antecedent rainfall to antecedent ambient air temperature. Multi-linear regression indicated that *E. coli* EMCs could be explained using rainfall intensity and the total number of sunlight hours in the day previous to the event across all four watersheds. Other factors showed high correlation with EMCs for individual watersheds, but the results were not applicable to all watersheds. Simple regression revealed a high correlation between the *E. coli* load and the sum of the storm rainfall intensities taken to the second power (called PIF_x). This parameter was paired with various other antecedent meteorological factors in a multiple regression. Antecedent total evaporation, average net radiation, total rainfall, and

average temperature where all significantly correlated to *E. coli* load when paired with the PIF_x variable.

The study by McCarthy et al. (2007) suggests that explanatory variables may be used to predict microorganism transport in urban environments. This is important in future efforts by the scientific community to model water quality in urban watersheds, but *E. coli* are not the only indicator bacteria used to assess the microbial quality of surface waters. Further study is needed to corroborate the results of McCarthy et al. (2007), and to relate other indicator organisms to meteorological and storm characteristics for application in municipalities which do not use *E. coli* as indicator bacteria.

Understanding how pollutants are transported is important in determining their fate in the environment. Transport of pollutants in urban environments is commonly characterized by the concept of the ”first flush.” This is the theory that during the initial part of the storm, pollutants that have built up during dry periods are flushed from a given watershed in higher concentrations and/or loads. This is often the justification behind stormwater BMP design guidance, as it is impossible to treat all the stormwater produced in a given watershed, but the highly concentrated first flush may be captured and treated. This concept has been explored for many pollutants and seems more pronounced for some pollutants and in some circumstances than others (Characklis and Weisner 1997, Line et al. 1996, Sansalone and Buchberger 1997, Sansalone and Cristina 2004,). Various methods have been used to examine the first flush in stormwater runoff. Analysis procedures commonly include either concentration or mass analysis to determine if a given pollutant is more prevalent in the first portion of the storm event.

Despite numerous studies on this topic, few peer reviewed journal articles have evaluated the first flush for microorganisms. A study conducted by CALTRANS (2000) explores the first flush for fecal coliform in highway land uses; however, only 8 storm events were captured primarily over a 6-week period. A concentration based approach was used in the study, whereby concentrations at the beginning of a given storm were compared to those later in the storm. The authors observed no strong first flush behavior. Details on the analysis methods used in this study are not entirely clear,

and a mass based approach in addition to the analyses performed would be valuable. A more in depth first flush study is detailed in McCarthy (2008). McCarthy explored the first flush for *E. coli* in four urban watersheds in Melbourne, Australia. McCarthy (2008) used a mass based approach, whereby the *E. coli* load in the first 30% of the total runoff was compared to the load produced by the remaining portion of the storm. The results of this study showed that the first flush was not consistent in all four watersheds. Further, explanatory variables were used to determine if correlating factors could be used to determine if a first flush would occur or not; however, common factors to all watersheds could not be found. No studies were found which evaluated the first flush for enterococci.

1.7 Treatment of Stormwater Contaminated by Indicator Bacteria

As stormwater is a source of indicator bacteria contamination, efforts are being made to treat runoff for indicator bacteria prior to its release into surface waters. In determining the potential that stormwater Best Management Practices (BMPs, also known as Stormwater Control Measures, or "SCMs") have for removing indicator bacteria, evaluating the treatment mechanisms utilized by these systems is critical. Theoretical microbial treatment mechanisms employed by the various types of stormwater BMPs are described in Table 1.2.

There are a number of treatment mechanisms commonly utilized in water treatment which are relevant to the removal and/or inactivation of microbes in stormwater runoff. Pathogens and indicator bacteria can exist in surface waters in either free form or attached to sediment (Characklis et al. 2005, Jeng et al. 2005, Fries et al. 2006, Krometis et al. 2007). Sedimentation of particle associated microbes in stormwater BMPs represents an opportunity for reductions in indicator bacteria in surface waters, although the size of the particle to which they are attached may determine the effectiveness of sedimentation (Jeng et al. 2005). Filtration and sorption may also act to sequester pathogens as stormwater passes through BMPs (Ferguson et al. 2003, Mankin 2007, Stevik et al. 2004). Filtration devices such as sand filters and bioretention areas are designed based on these mechanisms. Physical separation of the microbes occurs when they are obstructed from passage through small pore spaces in soils. Microbes may also have an electrical charge, which can lead to sorption to sediment particles (Sylvia et al. 2005). Exposure to sunlight, and thus UV

radiation, is also a powerful treatment mechanism (Canteras et al. 1995). UV radiation can damage DNA within microbes and lead to the death of the organism (Madigan et al. 2009). Last, predation by other organisms may act to remove microorganisms which are sequestered in BMPs (Ferguson et al. 2003).

Table 1.2: Common stormwater BMPs and theoretical pathogen removal mechanisms

(From Hathaway and Hunt, 2008)

BMP Type	Description	Treatment Mechanisms Relevant to Pathogen Removal
Dry detention basin	Fills during storm events, retains runoff for 1 to 2 days, and then slowly, but completely, drains. Remains dry between precipitation events. Primarily used for peak flow mitigation	Drying, sun exposure, sedimentation
Wet pond	Influent runoff theoretically replaces runoff captured from previous events (plug flow). Retains runoff for 1 or 2 days, and then slowly drains. Maintains water pool. Used for peak flow mitigation and some water quality improvement.	Sun exposure, sedimentation
Stormwater wetland	Fills during storm events, retains runoff for 1 or 2 days as it slowly drains. Maintains water pool. Has shallower water and more vegetation than wet pond. Normally used for water quality improvement, but can be used for peak flow mitigation.	Sun exposure, sedimentation, drying in shallow areas
Sand filter	Runoff first enters a sedimentation chamber before flowing through a column of soil. Sand chamber is dry between events.	Drying, sedimentation, filtration
Bioretention	Similar to sand filter, runoff enters system and passes through a soil media, where it is filtered. May pond 6 to 12 inches. Primarily a water quality BMP. System is dry between events.	Drying, sun exposure, sedimentation, filtration
Grassed swales	Runoff flows through an engineered, grassed channel used to convey it from one location to another.	Sedimentation, sun exposure, drying
Proprietary devices	Use baffles, settling chambers, filtration, and other means to separate floatable solids and promote sedimentation. Primarily intended for water quality.	Varies based on manufacturer: normally sedimentation and sometimes filtration

1.8 Removal in Stormwater BMPs

Although BMPs have been studied in detail for many pollutants, little peer-reviewed literature is available which documents their ability to remove or inactivate pathogens. BMPs are commonly constructed to facilitate removal of sediment, nutrients, and metals, but not indicator bacteria; however, indicator bacteria treatment mechanisms are present in these systems as discussed above. Theoretically, this should lead to some removal of indicator bacteria as stormwater passes through a given BMP. BMPs must be tested to evaluate how current design standards will perform with respect to this pollutant. Based on these evaluations, changes may be required to current design standards to facilitate sequestration and inactivation of indicator bacteria.

The majority of the BMP data associated with pathogen removal is available in a database format through the International Stormwater BMP Database (ISBD) (USEPA 2003b), a non-peer reviewed resource for stormwater professionals. Based primarily on data entered into the ISBD, the USEPA (2003b) concluded that BMP performance with respect to pathogens is less understood than for other pollutants. The data that have been collected have been primarily for sand filters, wetlands, and wet detention ponds. Further, the report highlights the variable performance that initial studies have shown with respect to BMP indicator bacteria removal.

1.8.1 Stormwater Wetlands and Wet Ponds

Although a number of studies have been performed on indicator bacteria removal in wetlands receiving wastewater (Karim et al. 2004; Vymazal 2004; Perkins and Hunter 2000; Ghermandi et al. 2007; Quiñónez-Diaz et al. 2001), few peer reviewed studies have been performed on indicator bacteria removal in wetlands receiving stormwater runoff. Birch et al. (2004) collected a limited number (4) of samples during high flow events at a stormwater wetland in Sydney, Australia, between April and June, 2000. The mean fecal coliform removal in the wetland was 76% with a range of 26 – 98%. The weighted average effluent fecal coliform concentration for the wetland was higher than the USEPA target value of 200 col/100ml for each of the 4 storms monitored.

Similar mean removal was found by Davies and Bavor (2000) in a study of a stormwater wetland receiving residential stormwater in New South Wales, Australia, from mid winter to early summer (July to December in Australia). Twenty-four samples were collected during this period on a weekly basis. The wetland mean removal of fecal coliform (called thermotolerant coliform in the study), enterococci, and heterotrophic bacteria was 79%, 85%, and 87%, respectively. The geometric mean effluent concentration of fecal coliform was 3600 col/100ml. A wet pond receiving residential stormwater runoff was also monitored in the study by Davies and Bavor (2000). Mean removal of fecal coliform (thermotolerant coliform), enterococci, and heterotrophic bacteria was -2.5%, 23%, and 22%, respectively. The authors noted that effluent concentrations often exceeded inflow concentrations, and the geometric mean effluent concentration of fecal coliform from the wet pond was 8100 col/100ml. Davies and Bavor (2000) associated the poor performance of the wet pond, relative to the wetland, to its poor removal of fine clay particles, to which the bacteria were "predominately absorbed." Samples were collected weekly from both the wetland and wet pond, but no breakdown was provided as to how many of the samples were taken during storm events. An analysis was performed to determine if rainfall in the 24 hours preceding a sample event was correlated to influent and effluent bacteria concentrations. Positive correlations were found for both the stormwater wetland and the wet pond for enterococci and fecal coliform at the inlet and for enterococci and heterotrophic bacteria at the outlet. Soil samples were taken from each site and, along with a sample from the water column, were analyzed for bacteria concentrations. Bacteria concentrations in the sediments were commonly several orders of magnitude higher than bacteria concentrations in the overlying water column.

Mallin et al. (2002) studied three wet ponds in Wilmington, North Carolina, from October 1997 to February 2000. The ponds were sampled monthly, regardless of whether the pond discharge was base flow or storm flow. The authors did not report the percentage of samples associated with wet weather. The average fecal coliform removal in the three ponds was 56%, 86%, and -13%. A similar correlation analysis was performed to that of Davies and Bavor (2000), and fecal coliform concentrations were positively correlated to rainfall occurring within 24 hours of a given pond being sampled. The geometric average effluent fecal coliform concentrations for the three wet ponds was 70, 43, and 85 col/100ml, respectively; however, only one of the wet ponds had an average influent

fecal coliform concentration higher than the USEPA targeted value of 200 col/100ml (488 col/100ml).

A more detailed study was performed by Struck et al. (2008) in an evaluation of indicator bacteria removal in wetlands and wet ponds using mesocosms. Results of the study showed indicator organism concentrations decreased exponentially over time after the mesocosms were dosed with stormwater which had been manipulated to increase bacterial concentrations. Struck et al. (2008) also examined factors contributing to this decay, indicating that temperature, light exposure, time, and other effects can impact indicator bacteria concentrations in simulated stormwater wetlands and wet ponds. Other effects included oxygen-reduction potential, pH, dissolved oxygen, and conductivity.

1.8.2 Bioretention and other Filtration Devices

Bioretention areas have not been studied in detail with respect to pathogens. Hunt et al. (2008) studied a bioretention area as part of the City of Charlotte Pilot BMP Program. The system was monitored with grab samples for both fecal coliform and *E. coli*, and showed reductions of 69 and 71%, respectively, from the inlet to the underdrain. The system consisted of a 4 foot deep, sandy loam soil media with approximately 5.7% silt and clay. The authors noted that the bioretention area drained relatively quickly, potentially leading to dry conditions within the cell and enhanced indicator bacteria removal byway of dessication.

Further bioretention research was provided via a column study by Rusciano and Obropta (2007). The researchers examined pathogen removal in simulated bioretention areas. The bioretention columns were loaded with diluted manure slurry with influent fecal coliform concentrations ranging from $2.3x10^7$ to $2.3x10^3$ CFU/100 ml. The average fecal coliform removal for 13 simulations over a 9 month period was approximately 96%, and leachate effluent mean concentrations ranged from $3.3x10^5$ to $2.0x10^1$.

Additionally, bioretention areas are expected to perform similarly to sand filters, as both are considered filtration-based BMPs. Austin-style sand filters were evaluated for fecal coliform removal

in a study by Barrett (2003). Five sand filters had a fecal coliform influent EMC of 11,200 and an effluent EMC of 3,900, a reduction of 65%.

1.8.3 Proprietary Devices

A proprietary device was evaluated for pathogen removal by Zhang and Lulla (2006). In this study, 2 hydrodynamic separation devices were studied in Providence, Rhode Island, for 12 storm events. Pathogen removal in systems 1 and 2 were determined to be 42% and 62%, respectively, for *E. coli* and 73% and 39%, respectively, for fecal coliform. The study noted that sediments within the device had higher concentrations of pathogens than the sump water, concluding that resuspension of pathogens from captured sediments could occur, potentially reducing removal efficiency below that reported. Additionally, Zhang and Lulla (2006) concluded that low BOD concentrations (less than 10 mg/L), and thus low nutrient concentrations, in the sump water of the device would make pathogen regeneration unlikely.

Due to the limited amount of literature pertaining to indicator bacteria removal by stormwater BMPs, more research is needed to aid communities throughout the United States in reaching their target indicator bacteria TMDLs. Determining which BMPs are capable of efficient indicator bacteria reduction will result in more effective watershed restoration programs. More specifically, if *E. coli* and enterococci become established as the most commonly utilized indicator bacteria, sequestration and removal of these indicator bacteria must be examined for a suite of stormwater BMP types, including wet ponds, dry detention, stormwater wetlands, bioretention, and proprietary devices.

1.9 Potential for Persistence in Stormwater BMPs

In addition to the removal mechanisms provided by stormwater BMPs, theoretically indicating their ability to remove indicator bacteria, each BMP also fosters a given set of environmental conditions. These conditions can influence the survival or die-off of microorganisms, and are variable depending on the BMP type. They include temperature, moisture conditions, pH, predation, and nutrient availability. These factors can impact the ability of microbes to persist in stormwater BMPs after they are captured through the removal mechanisms discussed above (USEPA 2001, Arnone and

Walling 2007, Stevik et al. 2004, Ferguson et al. 2003). Although various microbes have preferred environments, pathogen persistence is greater in moist, cool, dark environments which have a fairly neutral pH and are rich in nutrients (Stevik et al. 2004, Ferguson et al. 2003). In stormwater BMPs, this environment is sometimes present. Further, indicator bacteria persistence has been noted in stream sediments (Howell et al. 1996, Jamieson et al. 2004).

Although limited research has suggested their potential to remove indicator bacteria, stormwater BMPs may also be sources of these microbes. BMPs can attract wildlife such as domestic animals and waterfowl which can defecate in and around the BMPs, potentially resulting in increased pathogen loading. Likewise, pathogens and/or indicator bacteria sequestered in these systems through sedimentation could be resuspended during future stormwater events, and pathogens and/or indicator bacteria sorbed to soils may be stripped into effluent flows (Crabill et al. 1999, Fries et al. 2006, Stevik et al. 2004). Thus, removal mechanisms in stormwater BMPs are not well understood. It is unknown how well these mechanisms result in permanent removal of pathogens and indicator bacteria, or the degree to which conditions within BMPs could potentially lead to microbe persistence and reintroduction to surface waters.

1.10 References

Arnone, R.D. and J.P. Walling. (2007). "Waterborne pathogens in urban watersheds." *Journal of Water and Health*, 5(1), 149-162.

Barrett, M.E. (2003). "Performance, cost, and maintenance requirements of Austin sand filters." *Journal of Water Resources Planning and Management*, 129(3), 234-242.

Birch, G.F., C. Matthai, M.S. Fazeli, and J.Y. Suh. (2004). "Efficiency of a constructed wetland in removing contaminants from stormwater." *Wetlands*, 24(2), 459-466.

Booth, A.M., C. Hagedorn, A.K. Graves, S.C. Hagedorn, K.H. Mentz. (2003). "Sources of fecal pollution in Virginia's Blackwater River." *Journal of Environmental Engineering*, 129(6), 547 – 552.

Cahoon, L.B., J.C. Hales, E.S. Carey, S. Loucaides, K.R. Rowland, and J.E. Nearhoof. (2006). "Shellfish closures in southwest Brunswick County, North Carolina: septic tanks vs. storm-water runoff as fecal coliform sources." *Journal of Coastal Research*, 22(2), 319 – 327.

Canteras, J.C., J.A. Juanes, L. Perez, and K.N. Koev. (1995). "Modeling the coliforms inactivation rates in the Cantabrian Sea (Bay of Biscay) from in situ and laboratory determinations of T_{90}." *Water Science and Technology*, 32(2), 37 – 44.

Characklis, G.W. and M.R. Weisner. (1997). "Particles, metals, and water quality in runoff from large urban watershed." Journal of Environmental Engineering, 123(8), 753 – 759.

Characklis, G.W., M.J. Dilts, O.D. Simmons III, C.A. Likirdopulos, L.H, Krometis, M.D. Sobsey. (2005). "Microbial partitioning to settleable particles in stormwater." *Water Research*, 39, 1773 – 1782.

California Department of Transportation (CALTRANS). (2000). "First flush study 1999 – 2000 report." CTSW-RT-00-016.

Crabill, C., R. Donald, J. Snelling, R. Foust, and G. Southam. (1999). "The impacts of sediment fecal coliform reservoirs on seasonal water quality in Oak Creek, Arizona." *Water Research*, 33(9), 2163 – 2171.

Curriero, F.C., J.A. Patz, J.B. Rose, and S. Lele. (2001). "The association between extreme precipitation and waterborne disease outbreaks in the United States, 1948 – 1994." *American Journal of Public Health*, 91(8), 1194 – 1199.

Davies, C.M. and H.J. Bavor. (2000). "The fate of stormwater-associated bacteria in constructed wetland and water pollution control pond systems." *Journal of Applied Microbiology*, 89, 349-360.

Ferguson, C., A.M. de Roda Husman, N. Altavilla, D. Deere, and N. Ashbolt. (2003). "Fate and transport of surface water pathogens in watersheds." *Critical Reviews in Environmental Science and Technology*, 33(3), 299 – 361.

Fries, J.S., G.W. Characklis, and R.T. Noble. (2006). "Attachment of fecal indicator bacteria to particles in the Neuse River Estuary, N.C." *Journal of Environmental Engineering*, 132(10), 1338 – 1345.

Ghermandi, A., D. Bixio, P. Traverso, I. Cersosimo, and C. Thoeye. (2007). "The removal of pathogens in surface-flow constructed wetlands and its implications for water reuse." *Water Science and Technology*, 56(3), 207-216.

Graves, A.K., C. Hagedorn, A. Teetor, M. Mahal, A.M. Booth, R.B. Reneau. (2002). "Antibiotic resistance profiles to determine sources of fecal contamination in a rural Virginia watershed." *Journal of Environmental Quality*, 31, 1300 – 1308.

Haile, R.W., J. Witte, M. Gold, R. Cressey, C. McGee, R.C. Millikan, A. Glasser, N. Harawa, C. Ervin, P. Harmon, J. Harper, J. Dermand, J. Alamillo, K. Barrett, M. Nides, and G. Wang. (1999). "The health effects of swimming in ocean water contaminated by storm drain runoff." *Epidemiology*, 10(4), 355-363.

Hathaway, J.M., and W.F. Hunt. (2008). “Urban waterways: removal of pathogens in stormwater.” AGW-588-16-W, *North Carolina Cooperative Extension Service*, Raleigh, North Carolina.

Howell, J.M., M.S. Coyne, and P.L. Cornelius. (1996). “Effect of sediment particle size and temperature on fecal bacteria mortality rates and the fecal coliform / fecal streptococci ratio.” *Journal of Environmental Quality*, 25, 1216 – 1220.

Hunt, W.F., J.T. Smith, S.J. Jadlocki, J.M. Hathaway, and P.R. Eubanks. (2008). “Pollutant removal and peak flow mitigation by a bioretention cell in urban Charlotte, NC.” *Journal of Environmental Engineering*, 134(5), 403 – 408.

Jamieson, R.C., D.M. Joy, H. Lee, R. Kostaschuk, and R.J. Gordon. (2004). “Persistence of enteric bacteria in alluvial streams.” *Journal of Environmental Engineering and Science*, 3, 203 – 212.

Jeng, H.C., A.J. England, and H.B. Bradford. (2005). “Indicator organisms associated with stormwater suspended particles and estuarine sediment.” *Journal of Environmental Science and Health*, 40, 779 – 791.

Jones, M.P. and W.F. Hunt. (2009). “Bioretention impact on runoff temperature in trout sensitive waters.” *Journal of Environmental Engineering*, (accepted).

Karim, M.R., F.D. Manshadi, M.M. Karpiscak, and C.P. Gerba. (2004). “The persistence and removal of enteric pathogens in constructed wetlands.” *Water Research*, 38, 1831-1837.

Krometis, L.A., G.W. Characklis, O.D. Simmons III, M.J. Dilts, C.A. Likirdopulos, and M.D. Sobsey. (2007). “Intra-storm variability in microbial partitioning and microbial loading rates.” *Water Research*, 41(2), 506 – 516.

Line, D.E., J. Wu, J.A. Arnold, G.D. Jennings, and A.R. Rubin. (1997). “Water quality of first flush runoff from twenty industrial sites.” *Water Environment Research*, 69(3), 305 – 310.

Line, D.E., N.M. White, W.W. Kirby-Smith, and J.D. Potts. (2008). “Fecal coliform export from four coastal North Carolina areas.” *Journal of the American Water Resources Association*, 44(3), 606 – 617.

Madigan, M.T., J.M. Martinko, P.V. Dunlap, and D.P. Clark. (2009). “Brock biology of microorganisms.” 12th ed., *Pearson Education, Inc.*, San Francisco, California.

Mallin, M.A., K.E. Williams, E.C. Esham, and R.P. Lowe. (2000). “Effect of human development on bacteriological water quality in coastal watersheds.” *Ecological Applications*, 10(4), 1047-1056.

Mallin, M.A., S.H. Ensign, T.L. Wheeler, and D.B. Mayes. (2002). “Pollutant removal efficacy of three wet detention ponds.” *Journal of Environmental Quality*, 31, 654-660.

Mankin, K.R., L. Wang, S.L. Hutchinson, G.L. Marchin. (2007). *Transactions of the ASABE*, 50(4), 1159 – 1165.

McCarthy, D.T., V.G. Mitchell, A. Deletic, and C. Diaper. (2007). "*Escherichia coli* in urban stormwater: explaining their variability." *Water Science and Technology*, 56(11), 27-34.

McCarthy, D.T. (2008). "A traditional first flush assessment of *E. coli* in urban stormwater runoff." *International Conference on Urban Drainage*, Edinburgh, Scotland.

Myers, D.N., D.M. Stoeckel, R.N. Bushon, D.S. Francy, and A.M.G. Brady. (2007). "Fecal indicator bacteria." U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7, section 7.1

North Carolina Department of Environment and Natural Resources (NCDENR). (2008). "Phase II stormwater program." <http://h2o.enr.state.nc.us/su/NPDES_Phase_II_Stormwater_Program.htm> (November 29, 2008).

Perkins, J. and C. Hunter. (2000). "Removal of enteric bacteria in a surface flow constructed wetland in Yorkshire, England." *Water Research*, 34(6), 1941-1947.

Quiñónez-Diaz, M., M.M. Karpiscak, E.D. Ellman, and C.P. Gerba. (2001). "Removal of pathogenic and indicator microorganisms by a constructed wetland receiving untreated domestic wastewater."*Journal of Environmental Science and Health*, 36(7), 1311-1320.

Rusciano, G.M. and C.C. Obropta. (2007). "Bioretention column ctudy: fecal coliform and total suspended solids reductions." *Transactions of the ASABE*, 50(4), 1261-1269.

Sansalone, J.J. and S. G. Buchberger. (1997). "Partitioning and first flush of metals in urban roadway storm water." Journal *of Environmental Engineering*, 123(2), 134 – 143.

Sansalone, J.J. and C.M. Cristina. (2004). "First flush concepts for suspended and dissolved solids in small impervious watersheds." *Journal of Environmental Engineering*, 130(11), 1301 – 1314.

Schoonover, J.E. and B.G. Lockacy. (2006). "Land cover impacts on stream nutrients and fecal coliform in the lower piedmont of West Georgia." *Journal of Hydrology*, 331,371-382.

Stevik, T.K., K. Aa, G. Ausland, and J.F. Hanssen. (2004). "Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review." *Water Research*, 38, 1355 – 1367.

Struck, S.D., A. Selvakumar, and M. Borst. (2008). "Prediction of effluent quality from retention ponds and constructed wetlands for managing bacterial stressors in storm-water runoff." *Journal of Irrigation and Drainage Engineering*, 134(5), 567 – 578.

Sylvia, D.M., J.J. Fuhrmann, P.G. Hartel, and D.A. Zuberer. (2005). "Principles and applications of soil microbiology." 2nd ed., *Pearson Education, Inc.*, Upper Saddle River, New Jersey.

United States Environmental Protection Agency (USEPA). (1976). "Quality criteria for water." EPA-440/9-76-023, Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA). (1986). "Ambient water quality criteria for bacteria – 1986." EPA 440/5-84-002. Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA). (1999). "Preliminary data summary of urban storm water best management practices." EPA-821-R-99-012. Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA). (2001). "Protocol for developing pathogen TMDLs." EPA-841-R-00-002. Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA). (2003a). "Bacterial water quality standards for recreational waters: status report." EPA-823-R-03-008. Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA). (2003b). "Managing urban watershed pathogen contamination." EPA/600/R-03/111. Office of Research and Development, Cincinnati, OH.

United States Environmental Protection Agency (USEPA). (2008). "Water quality assessment and total maximum daily loads information (ATTAINS)."
< http://www.epa.gov/waters/ir/index.html> (November 29, 2008)

Viessman, W., and M. J. Hammer. (1998). "Water supply and pollution control." 6th ed., *Addison Wesley Longman, Inc.*, Menlo Park, California.

Vymazal, J. (2004). "Removal of enteric bacteria in constructed treatment wetlands with emergent macrophytes: a review." *Journal of Environmental Science and Health*, 40, 1355-1367.

Wade, T.J., N. Pai, J. Eisenberg, and J.M. Colford. (2003). "Do U.S. Environmental Protection Agency water quality guidelines for recreational waters prevent gastrointestinal illness? A systematic review and meta-analysis." *Environmental Health Perspectives*, 111(8), 1102-1109.

Weiskel, P.K., B.L. Howes, and G.R. Heufelder. (1996). "Coliform contamination of a coastal embayment: sources and transport pathways." *Environmental Science and Technology*, 30(6), 1872 – 1881.

Yacub, G.P., D.A. Castric, K.L. Stadterman-Knauer, M.J. Tobin, M. Blazina, T.N. Heineman, G.Y. Yee, L. Frazier. (2002). "Evaluation of Colilert and Enterolert defined substrate methodology for wastewater applications." *Water Environment Research*, 74(2), 131 – 135.

Young, K.D., and E.L. Thackston. (1999). “Housing density and bacterial loading in urban streams.” *Journal of Environmental Engineering*, 125, 1177-1180.

Zhang, X. and M. Lulla. (2006). “Evaluation of pathogenic indicator bacteria in structural best management practices.” *Journal of Environmental Science and Health Part A*, 41, 2483-2493.

2 Statistical Evaluation of Factors Affecting Indicator Bacteria in Urban Stormwater Runoff

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2.1 Abstract

An urban watershed in Raleigh, North Carolina, was monitored for indicator bacteria during 20 rain events. Results showed elevated levels of *E. coli*, enterococci, and fecal coliform. Samples were compared based on seasonality and were found to be statistically different ($p < 0.05$), with pairwise comparisons indicating significantly lower concentrations of *E. coli* and fecal coliform during the winter ($p < 0.05$). Enterococci concentrations were substantially lower in the winter and fall, but no significant differences were found between seasons during pairwise comparisons ($p < 0.05$). Correlation analyses showed multiple significant relationships between antecedent climate parameters, flow characteristics, and indicator bacteria concentrations. More detailed multiple linear regression yielded explanatory variables related to antecedent climate conditions. Variables were generally related to temperature and moisture conditions in the atmosphere and soil. The results of this study show indicator bacteria concentrations significantly vary based on season; however, this variability can partially be explained by antecedent climate data.

2.2 Introduction

Indicator bacteria are commonly used to denote the presence of fecal contamination in surface waters. Although there is some debate as to how well these bacteria relate to the presence of fecal pathogens, they offer a relatively inexpensive and expedient way to test the microbiological quality of surface waters (Arnone and Walling 2007). Various fecal indicator bacteria are currently used in the United States and elsewhere, including fecal coliform, *Escherichia coli* (*E. coli)*, and enterococci. In 1976, the United States Environmental Protection Agency (USEPA) set guidelines for indicator

bacteria concentrations in surface waters based on fecal coliform (USEPA 1976). Further study suggested *E. coli* and enterococci as better indicators of public health risk, leading to a change in USEPA recommendations in 1986 (USEPA 1986). Despite this change, fecal coliform is still used by many states (USEPA 2003), leading to the existence of large fecal coliform water quality data sets.

Studies have shown indicator bacteria concentrations in urban stormwater and streams under storm flow are commonly above regulatory levels for surface waters (Hathaway et al. 2009, Characklis et al. 2005, Krometis et al. 2009, Line et al. 2008). This represents a potential public health risk from fecal pathogens, as stormwater runoff can be conveyed to surface waters which act as recreational areas. Studies have shown increased health impacts to swimmers near stormwater outfalls in Santa Monica Bay, CA (Haile et al. 1999). Further, shellfish waters are sometimes closed for fishing after storm events due to elevated indicator bacteria concentrations, representing a loss of revenue in coastal areas (NCDENR 2009).

Total Maximum Daily Loads (TMDLs) are established for watersheds with fecal contamination. These TMDLs require basic modeling of targeted watersheds to determine sources and potential treatment opportunities. Until recent studies by McCarthy (2008), large indicator bacteria data sets containing multiple samples per storm event were uncommon for urban stormwater, making trend analysis difficult and limiting modeling efforts. Further, limited study has been performed to determine factors which influence indicator bacteria concentrations in urban environments. Identifying such factors is necessary to understand the mechanisms which drive indicator bacteria build up and transport in urban environments.

Microorganism survival can be affected by numerous environmental factors. These factors include temperature, moisture conditions, pH, predation, exposure to sunlight (UV radiation), and nutrient availability (USEPA 2001, Arnone and Walling 2007, Stevik et al. 2004, Ferguson et al. 2003). Environmental factors must be joined with hydrologic factors such as rainfall intensity and amount to further understand build-up and-wash off relationships.

At the watershed scale, a number of studies have evaluated correlating factors for indicator bacteria export from watersheds impacted by urbanization. However, many of these studies were performed in streams or estuaries (Kelsey et al. 2004, Young and Thackston 1999, Line et al. 2008, Schoonover and Lockaby 2006, Elder 1987, Eleria and Vogel 2005, Ferguson et al. 1996, Fries et al. 2006, Ortega et al. 2009, Mallin et al. 2000). Thus, while valuable information may be gleaned from these studies, the results are likely influenced by processes specific to streams or estuaries. Processes likely differ for indicator bacteria in urban stormwater runoff which has yet to enter a stream or estuary.

Various studies have correlated indicator bacteria in streams and estuaries to physical parameters such as land use, antecedent rainfall, discharge, rainfall depth, duration of storm event, intensity of storm event, and seasonality (Kelsey et al. 2004, Young and Thackston 1999, Line et al. 2008, Schoonover and Lockaby 2006, Elder 1987, Eleria and Vogel 2005, Ferguson et al. 1996). Water quality parameters such as salinity, water temperature, turbidity, pH, total suspended solids concentration, and various nutrients have also been related to indicator bacteria concentrations (Fries et al. 2006, Line et al. 2008, Ortega et al. 2009, Kelsey et al. 2004).

Recent studies by McCarthy (2008) and Selvakumar and Borst (2006) have specifically evaluated urban stormwater runoff. Selvakumar and Borst (2006) studied nine stormwater outfalls in Monmouth County, NJ, over fourteen storm events. At least seven storms were monitored for each outfall and tested for total coliforms, fecal coliforms, fecal streptococci, enterococci, *E. coli*, *Pseudomonas aeruginosa*, and *staphylococcus aureus*. Results of this study showed significant differences in all pathogens and indicator bacteria with season and significant differences with land use for all except *E. coli* ($p < 0.05$). Concentrations in the summer were not significantly different from fall and spring concentrations, and winter was determined to have the lowest concentrations. High density residential watersheds were found to have higher concentrations of bacteria than low density residential or landscaped commercial watersheds.

McCarthy (2008) evaluated *E. coli* concentrations in stormwater runoff from four urban watersheds in Melbourne, Australia. *E. coli* concentrations were correlated to various climate, hydrologic, and water quality variables, with a large number of variables being identified as significantly correlated.

Climate variables were averaged for antecedent periods of one day, two days, seven days, fourteen days, and twenty-eight days. Multiple linear regression was used to condense these relationships into a smaller number of explanatory variables. Multiple linear regression analyses were held to two total explanatory variables, one an antecedent climate variable and one either a flow or precipitation variable. Although selected variables changed from site to site, McCarthy (2008) identified a reduced model of average rainfall intensity and vapor pressure as significant for all four watersheds, although each variable was not significant within the reduced model for each watershed. The reduced model had R^2 values between 0.62 and 0.8 for the watersheds. Other reduced models found to be significant for at least one of the watersheds included variables such as maximum rainfall intensity, relative humidity, and air temperature. Other commonly used indicator bacteria species, fecal coliform and enterococci, were not evaluated. It should also be noted that the climate in Melbourne, Australia, varies from that of the Southeast United States, with less precipitation and smaller variations in temperature during the year.

The purpose of this study was to add to the limited understanding of indicator bacteria export from urbanized watersheds. Since indicator bacteria relationships may vary based on watershed characteristics and location, relationships proposed by previous researchers were explored, including seasonal variation and correlations between indicator bacteria types. Further, statistical analyses were used to explore relationships between all three commonly used indicator bacteria species, antecedent climate variables, and in-storm hydrologic variables. These analyses helped determine if responses to explanatory variables varied based on indicator bacteria type. Such differences may be important as the USEPA has suggested the use of enterococci as an indicator for marine waters and either *E. coli* or enterococci as an indicator for fresh waters (USEPA 1986). Understanding such relationships is important in developing TMDLs for impacted watersheds and in determining factors that must be considered when evaluating a stormwater Best Management Practice's (BMP) potential for indicator bacteria removal.

2.3 Materials and Methods

2.3.1 Site Description

The experimental watershed was located in Raleigh, NC, in a medium density residential neighborhood with approximately 35% imperviousness (Figure 2.1). An estimated 15% of the watershed was connected impervious area, primarily roadways. As is common in many residential neighborhoods in North Carolina, rooftops were typically not tied directly into the stormwater system. There were no stormwater BMPs installed in the watershed as it was developed prior to implementation of USEPA stormwater regulations. The watershed was approximately 5.1 ha (12.5 acres) with a mature tree canopy and geodetic coordinates (35.80^{o}N, 78.67^{o}W). Residents were commonly seen walking dogs during site visits. The stormwater and wastewater sewer systems were separate in the watershed, and sewer cross-connection was not expected as the stormwater outfall for the watershed was noted to be completely dry on multiple occasions during the late summer/early fall. During the rest of the year, base flow was noted, indicating possible groundwater intrusion into the stormwater system. The stormwater outfall was a 76-cm (30-inch) reinforced concrete pipe which fed a tributary to Beaverdam Branch.

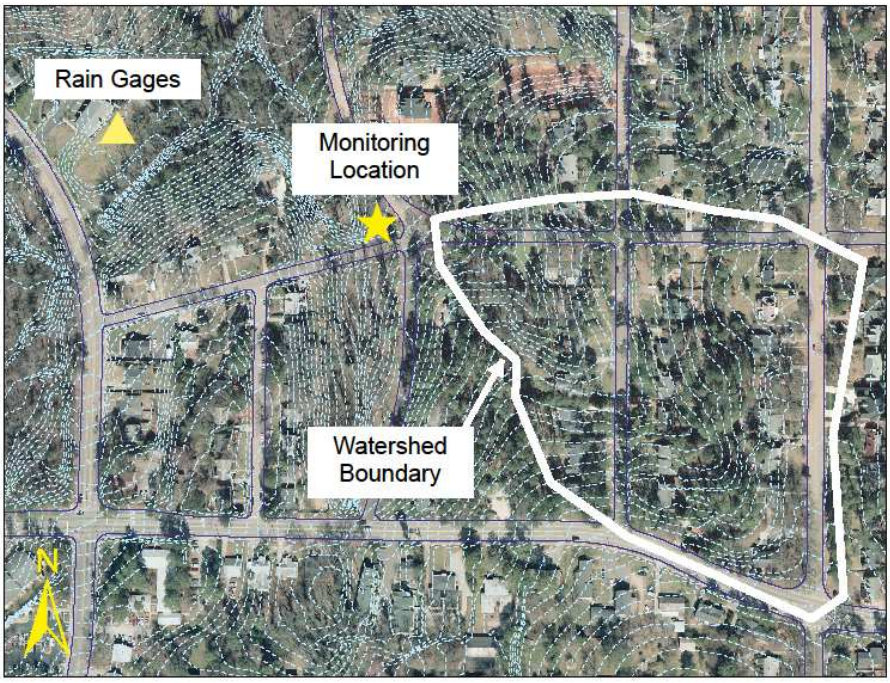

Figure 2.1: Arial Image of Experimental Watershed

2.3.2 Monitoring Methods

A compound weir was installed at the end of the culvert. Sufficient vertical distance was present between the weir invert and the receiving channel to avoid submerged conditions at the weir. An ISCO 730 bubbler module was used to record depth in the pipe. The depth was converted to flow using a stage-discharge relationship (Equation 1) developed for triangular-rectangular compound weirs by Jan et al. (2006).

$$Q = \frac{8}{15} C_{td} \sqrt{2g} \tan\left(\frac{\theta}{2}\right)\left(h_{2e}{}^{5/2} - h_{1e}{}^{5/2}\right) + \frac{2}{3} C_{rd} \sqrt{2g} \left(2b_1\right) h_1{}^{3/2} \quad (1)$$

Where: Q = flow in (m^3/s)

C_{td} = discharge coefficient for triangular, sharp-crested weir (0.58)

g = 9.81 m/s^2

θ = triangular weir angle (90^o)

h_{2e} = effective head above triangular weir invert (m)

h_{1e} = effective head above rectangular weir invert (m)

C_{rd} = discharge coefficient for rectangular weir (from Bos 1989)

b_1 = rectangular weir length on one side of triangular weir (m)

h_1 = head above rectangular weir (m)

The bubbler module was used in conjunction with an ISCO Avalanche refrigerated sampler which was equipped with a tray of 14 polypropylene bottles. Sampler intake tubing and bubbler tubing was fixed to the invert of the stormwater pipe. Evaluations by McCarthy et al. (2008b) indicated this collection point was not significantly different for indicator bacteria evaluations than those where samples were drawn from the top of the water column.

Prior to each anticipated storm, all bottles, pump tubing, and sampler tubing were washed, rinsed with deionized water, and autoclaved at 121^oC for 20 minutes to maintain sterility. Discrete, flow

paced samples were collected and distributed sequentially into the 14 bottles during storm events. Storm events were defined as any rainfall event which produced runoff in excess of base flow during which 5 samples could be collected. All events exceeded 0.4 cm. Flow pacing was manipulated prior to and during the storm to achieve an adequate characterization of the storm. If adjustments were required, flow pacing was increased as the storm progressed to allow the greatest resolution during the initial portion of the storm when flow rates and concentrations were expected to have the highest variability. Although base flow was not present in the stormwater outfall during the entire study, base flow samples were collected from the stormwater outfall on 5 occasions. Care was taken not to disturb any sediment in the bottom of the pipe during these base flow sample events. Base flow samples were used to gain additional information about the system, but were not used in any analyses. Stormwater samples were collected from the monitoring location and transported to the Department of Biological and Agricultural Engineering at North Carolina State University where they were refrigerated until analyzed.

A tipping bucket rain gage was installed approximately 190 m (630 ft) from the watershed outfall and 560 m (1850 ft) from the outer boundary of the watershed (Figure 2.1). A HOBO data logger stored data from the tipping bucket rain gage and a manual rain gage was placed on site to verify precipitation depths. Data were used to generate depth and intensity values for rainfall events. Additional climate data were obtained from a weather station at the Lake Wheeler Road Field Laboratory located approximately 8.3 km (5.2 mi) from the experimental watershed. The weather station is operated by the North Carolina Agricultural Research Service. Climate data preceding the storm were averaged at various time intervals to establish antecedent conditions. Data were averaged for the 1, 2, 7, 14, and 28 days prior to a given storm event, similar to the methodology employed by McCarthy (2008). Climate data which were used for correlation analysis were air temperature, relative humidity, vapor pressure, solar radiation, potential evapotranspiration (PET), and precipitation total. Vapor pressure was not collected at the Lake Wheeler Road Field Laboratory. Thus, it was calculated using standard equations (NOAA 2009). Historical average temperature and precipitation data for Raleigh, NC, are presented in Table 2.1.

Table 2.1: Historical Climate data for Raleigh, NC, from 1973-2000 (SCONC 2009)

Parameter	Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sept	Oct	Nov	Dec
Average Daily Maximum Temperature (^{o}C)	9.33	11.7	16.2	21.4	25.3	29.1	31.1	29.9	26.7	21	16.3	11.2
Average Daily Minimum Temperature (^{o}C)	-1.1	0.17	4.33	8.78	13.7	18.3	20.8	20.1	16.6	9.67	5.33	0.83
Average Total Rainfall (cm)	11.3	8.97	11.3	7.57	10.2	10.3	11	10.9	10.8	9.6	7.77	8.15

2.3.3 Sample Analysis

Bacteria analyses were performed within 24 hours of sample collection. Fecal coliform and *E. coli* were enumerated using Colilert (defined substrate technologies; IDEXX, Westbrook, Maine). The Colilert method was modified to detect fecal coliform and *E. coli* by incubating the samples at 37^{o}C for 1 to 3 hours followed by incubation at 44.5^{o}C for 21 to 23 hours (Yakub et al. 2002). Enterococci were enumerated using Enterolert (defined substrate technologies; IDEXX, Westbrook, Maine). The Enterolert method was performed per manufacturer guidelines by incubating at 41^{o}C for 24 hours. Positive (stock culture) and negative (dilution blank) controls were used during laboratory analyses, although enterococci standards were not available until the latter two thirds of the study. Samples typically required either a 100:1 or 1000:1 dilution due to high bacteria counts. Base flow samples were only tested for *E. coli* and fecal coliform to examine the potential presence of sewer cross connections.

Further sample analysis was performed at the North Carolina Center for Applied Aquatic Ecology (NCCAAE). An aliquot was taken from each discrete sample, composited in an acidified bottle, sent to NCCAAE, and tested for TKN using the EPA 351.2 method (USEPA 1983). The remainder of each discrete sample was tested for total suspended solids (TSS) analysis using SM 2540D (APHA 1998).

2.3.4 Data Analysis

To estimate loading for a given storm, discrete sample concentrations for indicator bacteria and TSS were multiplied by the volume corresponding to the sample (equation 2). Discrete samples which

exceeded the maximum detectable concentration for the analysis were not included in loads analysis.

$$Load = \sum_{i=1}^{n} c_i V_i \qquad (2)$$

Where:

c_i = concentration at time i

V_i = volume of runoff during time i

Loads were then divided by the total volume of stormwater produced by a given storm to generate an Event Mean Concentration (EMC) for each storm (equation 3 – USEPA 2002).

$$EMC = \frac{\sum_{i=1}^{n} c_i V_i}{\sum_{i=1}^{n} V_i} \qquad (3)$$

2.3.5 Statistical Analysis

All statistical analyses were performed using SAS 9.1 (SAS 2001). Indicator bacteria were used as dependent variables; thus, they were checked for normality using histograms and a Kolmogorov-Smirnov test (Hollander and Wolfe, 1999). Indicator bacteria EMCs were found to be log-normally distributed and were used in this format throughout the statistical analyses. All statistical analyses were performed at an alpha = 0.05 significance level unless otherwise noted.

Seasonal variations in indicator bacteria concentration were first explored with a distribution free Kruskal-Wallis test to determine if there were any significant differences among all seasons (Hollander and Wolfe, 1999). Pairwise analyses were then performed between seasons using a

distribution free Wilcoxon Rank Sum test (Hollander and Wolfe, 1999). This additional analysis allowed comparisons between all combinations of seasonal indicator bacteria concentrations.

A distribution free Spearman rank correlation test was used to explore correlations between indicator bacteria EMCs and climate, flow, and precipitation variables (Hollander and Wolfe, 1999). Using the PROC CORR procedure in SAS 9.1, both a Spearman's rank correlation coefficient (ρ) and a p-value were generated. Thus, any correlation could be verified for statistical significance.

To determine which flow, rainfall, or antecedent climate variables best explained the variability of indicator bacteria concentrations in urban watersheds, multiple linear regression analyses were used. Multiple linear regression analyses utilized the STEPWISE selection procedure in the PROC REG function of SAS 9.1. This procedure generally involves adding variables to the model piecemeal in order of largest F statistic. Due to the large number of predictor variables being considered in the analysis, only the first three variables selected by the procedure were used as explanatory variables as to not overparameterize the model. After the selection procedure, the three selected variables were placed in a model and evaluated using the PROC REG function. Variance Inflation Factors (VIF) were generated to ensure multicollinearity was not a problem among the predictor variables (Ott and Longnecker, 2001). Further, residuals were plotted and checked for normality to assure that the assumptions of the model were not violated.

2.4 Results and Discussion

2.4.1 Summary Statistics

Between October, 2008, and September, 2009, twenty storm events were monitored. Storms ranged in size from 0.41 to 5.6 cm (0.16 to 2.2 inches). Five events were monitored for each of the four seasons during the period. At least 5 discrete samples were collected during each event. On average, ten discrete samples were collected per event (Figure 2.2). TSS was evaluated for each discrete sample for thirteen of the sample events. TSS was not evaluated until later in the study, so the fall season was not represented by these samples. TKN concentrations were measured for 16 rain events. Again, the fall season was not well represented for this parameter.

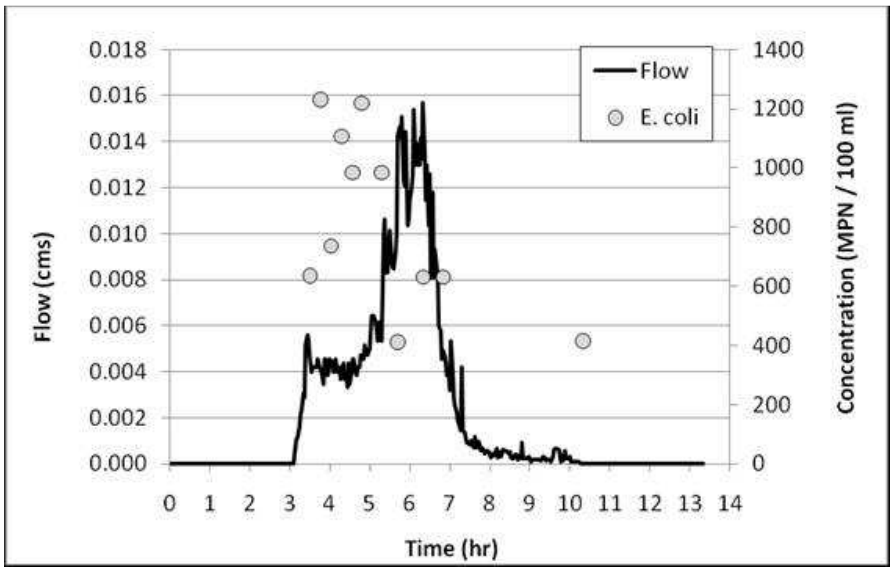

Figure 2.2: Example of sample collection spacing during rain event on 2/18/2009

Concentrations for each storm and summary statistics are provided in Table 2.2. EMCs for *E. coli* ranged from 700 to 84,700 MPN / 100 ml (MPN = Most Probable Number); EMCs for fecal coliform ranged from 1500 to 342,400 MPN / 100 ml; and EMCs for enterococci ranged from 1300 to 181,800 MPN / 100 ml. Although the maximum EMC for each indicator bacteria was high, McCarthy (2008) showed similar maximum *E. coli* concentrations for two of four watersheds in Melbourne, Australia.

Base flow samples had substantially lower concentrations of indicator bacteria than those collected during rain events. Fecal coliform concentrations ranged from 204 to 2890 MPN / 100 ml with a median concentration of 730 MPN / 100 ml. *E. coli* concentrations ranged from < 10 to 626 MPN / 100 ml with a median concentration of 136 MPN / 100 ml. These numbers compare well to studies of indicator bacteria concentrations in drainage entering stormwater BMPs during dry conditions (Krometis et al. 2009) and to average concentrations of indicator bacteria in stream base flow (Characklis et al. 2005). Relatively low base flow concentrations compared to those during storm events, combined with completely dry conditions in the stormwater outfall during some periods of the study, suggests a lack of sewer cross-connections in the stormwater system. However, base flow

is likely due to groundwater intrusion into the pipe system, which could potentially be influenced by groundwater contaminated with fecal indicator bacteria from leaking sewer lines.

Table 2.2: Indicator bacteria, TSS, and TKN concentrations for each storm

Date	Season	Rain (cm)	Number of Samples Collected	*E. coli* EMC (MPN / 100 ml)	Fecal Coliform EMC (MPN / 100 ml)	Enterococci EMC (MPN / 100 ml)	TSS EMC (mg/ L)	TKN (mg/L)
10/17/2008	Fall	2.03	14	32,483	134,175	2,682	.	.
11/4/2008	Fall	2.69	16	16,539	46,186	3,225	.	.
11/14/2008	Fall	2.82	13	12,491	72,199	13,504	.	.
11/25/2008	Fall	0.95	5	3,475	14,623	5,111	.	.
12/20/2008	Fall	2.97	9	10,943	25,695	11,179	.	2.38
1/6/2009	Winter	2.55	7	4,653	7,564	14,373	.	0.82
1/28/2009	Winter	1.24	11	8,913	14,115	6,568	.	5.10
2/11/2009	Winter	0.41	8	13,480	18,009	2,728	309	6.94
2/18/2009	Winter	1.70	11	710	1,469	1,306	87	1.84
3/13/2009	Winter	2.11	10	8,806	13,145	7,687	106	3.71
3/26/2009	Spring	1.80	8	12,868	17,024	4,261	309	4.22
4/2/2009	Spring	0.94	5	46,157	98,350	50,503	196	2.66
5/8/2009	Spring	1.85	10[a]	84,688	165,032	44,229	160	2.12
5/14/2009	Spring	0.56	7[b]	43,965	96,248	181,846	125	2.66
6/4/2009	Spring	3.94	12	59,302	113,567	30,371	122	2.71
7/17/2009	Summer	1.40	10	26,882	185,230	29,181	181	2.99
7/25/2009	Summer	0.41	6	4,487	63,327	3,175	44	2.28
8/5/2009	Summer	1.65	12	74,658	342,405	53,633	97	1.77
8/28/2009	Summer	1.50	12	29,081	118,925	20,962	49	2.44
9/7/2009	Summer	5.59	18	18,280	55,558	16,566	33	1.21
Arithmetic Mean =		1.96	10.2	25,643	80,142	25,155	140	2.87
Median =		1.75	10.0	15,010	59,442	12,342	122	2.55
Standard Deviation =		1.25	3.5	24,323	82,931	40,380	90	1.52

a. Note: only 9 enterococci samples
b. Note: only 5 enterococci samples

2.4.2 Seasonal Variation

Seasonal variations in indicator bacteria are shown in Figure 2.3. All indicator bacteria had lower concentrations during the winter; however, this was less pronounced for enterococci. The highest average *E. coli* and enterococci concentrations were observed during the spring, while the highest fecal coliform concentrations were observed during the summer.

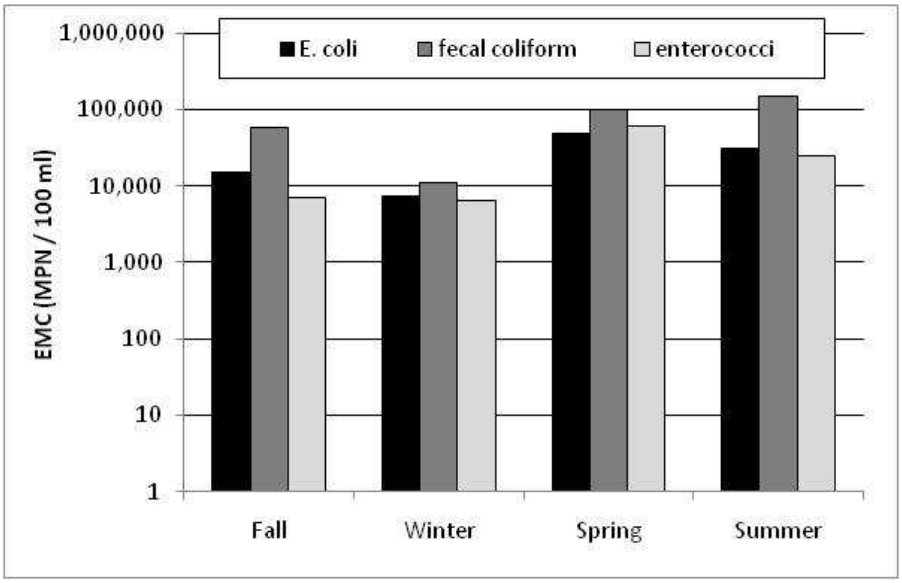

Figure 2.3: Average seasonal EMC for each indicator bacteria

The results of the Kruskal-Wallis test indicated a seasonal difference in EMC for *E. coli*, fecal coliform, and enterococci. The Wilcoxon Rank Sum tests allowed detailed investigation of pairwise differences between all seasons for each indicator bacteria. For *E. coli*, significant differences were found between winter - spring and fall - spring. For fecal coliform, fall - winter, winter - spring, and winter - summer were all significantly different. Although the Kruskal-Wallis test indicated seasonal differences for enterococci, none of the pairwise comparisons was statistically significant.

Winter concentrations were consistently associated with significant differences among indicator bacteria. These data are similar to observations made by Selvakumar and Borst (2006), Line et al. (2008), Young and Thackston (1999), and Schoonover and Lockaby (2006), who noted lower

concentrations of indicator bacteria in surface waters during the winter. The results of the seasonal analysis are presented in Table 2.3.

Table 2.3: Analysis of seasonal differences in indicator bacteria concentrations

Indicator Bacteria	Kruskal-Wallis (p-values)	Wilcoxon Rank Sum (p-values)					
		Seasonal Pair Evaluated					
		fall - winter	fall - spring	fall - summer	winter - spring	winter - summer	spring - summer
E. coli	***0.026***	0.1745	***0.0283***	0.2506	***0.0163***	0.0758	0.2506
fecal coliform	***0.0097***	***0.0163***	0.2506	0.1172	***0.0163***	***0.0090***	0.4647
enterococci	***0.0412***	1.0000	0.0556	0.0952	0.0556	0.0556	0.3095

Note: Significant relationships are bold and italicized

2.4.3 Correlation Analysis

Correlations among indicator bacteria are shown in Table 2.4. Fecal coliform and *E. coli* were enumerated concurrently using the Colilert method and thus were not analyzed for correlation due to concerns over independence of the data. Correlations between enterococci and the other indicator species were statistically significant. Enterococci correlations to *E. coli* and fecal coliform had Spearman coefficients of 0.68 and 0.58, respectively.

Studies by Ortega et al. (2009) and Fries et al. (2006) showed poor correlations between various indicators in estuaries. However, a study of indicator bacteria in urban stormwater runoff by Selvakumar and Borst (2006) showed high correlations between fecal coliform and *E. coli* (Pearson correlation coefficient of 0.771), moderate correlations between *E. coli* and enterococci (Pearson correlation coefficient of 0.425), and moderate correlations between fecal coliform and enterococci (Pearson correlation coefficient of 0.534). Although these relationships are expected to be variable between watersheds, it may be possible to monitor both *E. coli* and fecal coliform for a period of time to correlate these two indicators for a given watershed. This would aid in using fecal coliform datasets when developing TMDLs based on *E. coli* (provided bacteria sources have not significantly changed since the data were collected). Such analysis was performed in a case study for Lower Geddes Pond in Michigan as presented by USEPA (2001).The same correlations may not be possible;

however, when fecal coliform data are desired for use in enterococci based TMDLs due to relatively low correlations noted in this study and in Selvakumar and Borst (2006).

Table 2.4: Correlations between indicator bacteria

Indicator Bacteria	enterococci	
	ρ	p-value
E. coli	0.68	0.001
fecal coliform	0.58	0.008

TSS and TKN were also analyzed for correlations with indicator bacteria. However, very poor correlations were found, none of which were statistically significant. Spearman coefficients for correlation between TSS and *E. coli*, fecal coliform, and enterococci were 0.203, 0.022, and 0.132, respectively. Spearman coefficients for correlation between TKN and *E. coli*, fecal coliform, and enterococci were 0, -0.097, and -0.229, respectively.

Results for studies which correlated indicator bacteria and TSS have been variable. McCarthy et al. (2007) showed significant correlations for TSS and *E. coli* for two of four watersheds studied in Melbourne, Australia ($p < 0.05$). However, studies of surface waters by Fries et al. (2006) and Line et al. (2008) showed poor correlation between indicator bacteria and TSS concentrations. Although it is understood that indicator bacteria attach to and are transported with particles (Characklis et al. 2005, Krometis et al. 2007, Fries et al. 2006), TSS concentrations should not be used to infer microbiological quality in surface waters.

Although TKN would intuitively be linked to indicator bacteria, due to the potential of similar sources for the two parameters, studies have shown varied correlations between nitrogen species and indicator bacteria. A study by Line et al. (2008) showed no correlation between fecal coliform concentrations and NO_3-N or NH_3-N in three watersheds in North Carolina. Conversely, McCarthy (2008) showed positive correlations between NH_3-N and *E. coli* for 3 out or 4 watersheds monitored in Melbourne, Australia. Due to varied conclusions regarding correlations between nitrogen species and indicator bacteria, there does not seem to be a clear interaction.

Numerous significant correlations were found between each indicator bacteria and various hydrologic and antecedent climate variables (Table 2.5). Despite the inclusion of multiple flow and precipitation metrics, only peak flow was found to be significantly correlated to indicator bacteria EMCs. However, peak flow is directly related to rainfall intensity, thus signifying the importance of rainfall and flow characteristics in bacteria transport in urban watersheds. Antecedent rainfall conditions likely impact both the amount of indicator bacteria build-up and the amount of moisture present in a watershed, but this was only statistically true for enterococci in this correlation analysis. It should be noted that enterococci consistently showed poorer correlation to explanatory variables.

For any given variable, it was common to have multiple antecedent periods of time correlated to bacteria concentrations. For example, air temperature was significantly correlated to all three indicator bacteria when averaged for 1, 2, 7, 14, and 28 days before the storm event. This is likely due to a given climate variable not changing substantially over a 28 day period.

Temperature and vapor pressure consistently provided significant correlations with all indicator bacteria, similar to relationships found by McCarthy (2008). Various studies have shown indicator bacteria concentrations in surface waters are higher during warmer parts of the year (Selvakumar and Borst 2006, McCarthy 2008, Young and Thackston 1999, Line et al. 2008, Schoonover and Lockaby 2006). The strong positive correlation between temperature and indicator bacteria concentration is somewhat unexpected as many studies have shown that die off rates for indicator bacteria are higher as temperature increases (Kibbey et al. 1978, Van Donsel et al. 1967, Ferguson et al. 2003, Crane and Moore 1986). However, interactive effects between such factors as temperature and moisture may result in more complicated relationships (Wang et al. 2004, Kibbey et al. 1978).

This paradox has been examined by McCarthy et al (2008), Crane and Moore (1986), and Tiefenthaler et al. (2009). Based on the conclusions of these and other studies, the possible explanations for the increase in indicator bacteria concentration with increased temperatures include: (1) Increased sources of indicator bacteria due to domestic and wild animal activity and (2) increased persistence due to seasonal variations in environmental conditions such as temperature, humidity, and rainfall patterns. Essentially, indicator bacteria die-off is likely based on a combination

of factors which vary from season to season (Crane and Moore, 1986). Temperature probably acts as a surrogate for such seasonal variations and interactions in this analysis.

Table 2.5: Results of correlation analysis (only significant relationships shown)

Variable	*E. coli*		fecal coliform		enterococci	
	ρ	p-value	ρ	p-value	ρ	p-value
peak flow	0.4842	0.0305	0.5699	0.0087	0.5444	0.0131
antecedent dry period	-	-	-	-	-0.4737	0.0349
Rain Total 7 days	-	-	-	-	0.6102	0.0043
Rain Total 14 days	-	-	-	-	0.5790	0.0075
Rain Total 28 days	-	-	-	-	0.5323	0.0157
Air Temperature 1 day	0.6511	0.0019	0.8316	<.0001	0.5053	0.0231
Air Temperature 2 days	0.6767	0.0011	0.8346	<.0001	0.5158	0.0199
Air Temperature 7 days	0.6346	0.0027	0.8241	<.0001	0.5173	0.0195
Air Temperature 14 days	0.6000	0.0052	0.8226	<.0001	0.5368	0.0147
Air Temperature 28 days	0.5624	0.0098	0.8000	<.0001	0.5098	0.0217
Vapor Pressure 1 day	0.6571	0.0016	0.8812	<.0001	0.5774	0.0077
Vapor Pressure 2 days	0.6421	0.0023	0.8346	<.0001	0.5489	0.0122
Vapor Pressure 7 days	0.6541	0.0018	0.8481	<.0001	0.5805	0.0073
Vapor Pressure 14 days	0.5624	0.0098	0.8030	<.0001	0.5639	0.0096
Vapor Pressure 28 days	0.5609	0.0101	0.7774	<.0001	0.5143	0.0203
PET 1 day	0.5323	0.0157	0.6105	0.0042	-	-
PET 2 days	0.4993	0.0250	0.6165	0.0038	-	-
PET 7 days	0.4556	0.0435	0.4947	0.0266	-	-
PET 14 days	0.6311	0.0028	0.7183	0.0004	0.5386	0.0143
PET 28 days	0.6030	0.0049	0.7729	<.0001	0.5203	0.0187
Relative Humidity 7 days	0.6135	0.0040	0.7744	<.0001	0.5203	0.0187
Relative Humidity 14 days	0.5218	0.0183	0.6977	0.0006	0.4602	0.0412
Relative Humidity 28 days	-	-	0.4626	0.0400	-	-
Solar Radiation 2 days	-	-	0.5038	0.0235	-	-
Solar Radiation 14 days	0.6000	0.0052	0.6301	0.0029	0.4872	0.0293
Solar Radiation 28 days	0.5895	0.0062	0.6797	0.0010	0.5068	0.0226

Other contradictory relationships were observed in the correlation analysis. PET consistently had a positive correlation to indicator bacteria concentrations. This was unexpected, as greater desiccation was expected as evaporation within the watershed increased. However, correlation analysis between air temperature and PET showed a strong relationship, indicating that as air temperature increases, so too does PET. Thus, the true affect of PET may not be illustrated in the data, as it is overwhelmed by that of other factors. Air temperature is also related to vapor pressure, relative humidity, and solar radiation. All would be expected to increase during the warmer months in the Southeastern United States.

2.4.4 Multiple linear Regression

The multiple linear regression analysis condensed the results of the correlation analysis into a smaller number of explanatory variables that best described the indicator bacteria concentrations. Final reduced models showed VIFs for all variables were less than 10, indicating little autocorrelation among the selected variables (Ott and Longnecker 2001). This was of particular importance given the relationship between temperature and many other climate variables. The reduced models for each indicator bacteria are presented in Table 2.6.

Table 2.6: Results of multiple linear regression analysis

Indicator Bacteria	Variable 1			Variable 2			Variable 3			Overall (R^2)
	name	VIF	p	name	VIF	p	name	VIF	p	
E. coli	2 day average air temperature	1.01	<.0001	28 day total rain	1.01	0.0059	2 day total rain	1.02	0.0078	0.7462
fecal coliform	2 day average air temperature	2.00	0.0058	14 day average relative humidity	1.95	0.0335	7 day total rain	1.09	0.0555	0.802
enterococci	7 day total rain	1.06	0.0094	14 day average relative humidity	1.06	0.0275	-	-	-	0.526

The reduced model for *E. coli* included the average air temperature and total rain amount for 2 days preceding the rain event, and the total rain amount for the 28 days preceding the event. The

coefficient of determination (R^2) for this model was 0.75, with each variable being significant in the model. The parameter estimate for total rain for 2 days preceding an event was negative, indicating that if rain is occurring frequently in the days preceding a given storm, the indicator bacteria source in the watershed is reduced by wash-off dynamics. Conversely, the total rain amount for 28 days preceding the event is likely an indication of how wet the watershed is, but not necessarily whether indicator species have been subject to wash-off. Moist soils would be expected to facilitate slower die-off of indicator bacteria (Kibbey et al. 1978).

The reduced model for fecal coliform included the antecedent 2 day average air temperature, the antecedent 7 day total rainfall, and antecedent 14 day relative humidity. The coefficient of determination (R^2) for this model was 0.80, and only the antecedent 7 day total rainfall had a p-value above 0.05 ($p = 0.055$). Parameter estimates for each variable were positive, indicating that each variable leads to an increase in fecal coliform concentrations. Thus, these variables all contribute to the build-up/persistence of fecal coliform in the watershed. Specifically, antecedent 7 day total rainfall and 14 day average relative humidity seem to relate to the amount of moisture in the watershed and atmosphere leading up to the event. Atmospheric moisture was also considered important in evaluations of *E. coli* export from urban watersheds in Melbourne, Australia (McCarthy 2008).

The reduced model for enterococci included the total 7-day antecedent rainfall total and the antecedent 14-day average relative humidity. Both of these variables were also selected for the fecal coliform reduced model; however, no other variables were selected by the model based on the default SAS significance threshold of $p < 0.15$. The coefficient of determination (R^2) for this model was 0.53. As with fecal coliform, all variables in the reduced model seem related to build-up/persistence of the enterococci in the watershed, and atmospheric and/or soil moisture seem to be important factors. Statistical modeling of enterococci yielded lower R^2 values than *E. coli* or fecal coliform. Generally, enterococci are considered to have a slower die off rate than *E. coli* and fecal coliform in the environment (USEPA 2001). Thus, slight climate variations may have less of an impact on enterococci or may be harder to detect.

From these data, it seems antecedent conditions do have an impact on indicator bacteria and may help explain the variability seen in concentrations of indicator bacteria in urban watersheds. Similar conclusions were made by McCarthy (2008). However, selected variables indicate high complexity for indicator bacteria in urban watersheds. Simple linear regression of one antecedent climate variable is not sufficient for indicator bacteria modeling (McCarthy et al. 2007). In general, variables selected for the models were related to antecedent climate instead of storm-specific hydrologic or rainfall characteristics. Commonly included were variables that could be related to differences in atmospheric and soil moisture, such as total rain preceding the event and relative humidity. Antecedent rainfall totals were also found to influence indicator bacteria concentrations in Murrells Inlet in South Carolina by Kelsey et al. (2004). Temperature was also important for *E. coli* and fecal coliform; however, temperature possibly acted as a surrogate for changes in the watershed associated with seasonal differences.

2.5 Conclusions

Flow weighted stormwater samples were collected for 20 events in a medium density residential neighborhood in Raleigh, North Carolina. *E. coli* and fecal coliform concentrations were significantly lower during winter storm events ($p < 0.05$). Enterococci concentrations during the winter and fall were also lower, but the differences were not statistically significant ($p < 0.05$).

Correlation analysis showed numerous significant relationships between indicator bacteria concentrations, antecedent climate variables, and flow variables. Simple correlation analysis appeared to misconstrue the effect of climate variables on indicator bacteria concentrations. Many relationships that appeared during the correlation analysis were not logical and were likely the result of multicollinearity between variables.

A multiple linear regression analysis allowed a more detailed examination of these relationships. Temperature and variables related to soil and atmospheric moisture appeared to be important in explaining the variability of indicator bacteria concentrations. All three indicator bacteria seemed to show similar behavior in regard to antecedent climate based on the variables selected by the multiple linear regression. However, statistical models for enterococci were not as predictive.

Enterococci are generally regarded as more persistent in the environment (USEPA 2001). Thus, temporal climate variations may be harder to associate to enteroccocus concentrations. Therefore, caution should be taken when applying modeling techniques from one indicator bacteria to another.

Although watershed studies can provide useful observations of microbial transport and fate, variable relationships likely exist based on watershed characteristics. Further, transport mechanisms within urban stormwater conveyances are presumably different than those in lotic systems; thus, care should be taken when extrapolating between data collected from stormwater outfalls and data collected within streams or estuaries. The intent of this analysis was to determine important climatic variables for this watershed and to compare those variables to those determined for other watersheds within scientific literature. The reduced models provided in this analysis are unlikely to be applicable to other watersheds with adequate confidence. However, as discussed herein, common relationships were identified which will aid the scientific community in the continued development of microbial transport and fate models. In particular, climate does appear to influence indicator bacteria concentrations in stormwater runoff from urban watersheds. Process-based approaches will ultimately be required to develop models which are robust with respect to watershed location and characteristics.

The results of this study have multiple implications for watershed management:

(1) Indicator bacteria exported via urban stormwater can be a substantial source of non-point pollution in watersheds. Based on the magnitude of indicator bacteria concentrations, stormwater runoff should be carefully considered in TMDLs.
(2) Per USEPA guidance, TMDLs must account for seasonal variations in indicator bacteria. As noted in this and other studies, these variations can be significant and should be carefully considered.
(3) Stormwater best management practices should be evaluated for differences in performance based on season. Poor performance during warmer months, combined with high influent concentrations, could make watershed restoration efforts which employ these practices of reduced benefit. This is of particular concern during warm months when recreational use of surface waters is high.

(4) Antecedent climate conditions can explain some of the variability noted for indicator bacteria concentrations in urban stormwater. Such relationships seem complex and likely will require incorporation of many variables. Atmospheric and soil moisture conditions appear important at the watershed scale, which is intuitive based on the impact of moisture on indicator bacteria in laboratory studies. However, further understanding of these relationships would result in more efficient management of recreational waters.

2.6 Acknowledgements

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2.7 References

American Public Health Association, American Water Works Association, and Water Environment Federation (APHA, AWWA, and WEF). (1998). "Standard methods for the examination of water and wastewater." 20th edition. American Public Health Association, Alexandria, VA.

Arnone, R.D. and J.P. Walling. (2007). "Waterborne pathogens in urban watersheds." *Journal of Water and Health*, 5(1): 149-162.

Bos, M.G. (1989). "Discharge measurement structures."3rd edition. International Institute for Land Reclamation and Improvement, Wageningen, The Netherlands.

Characklis, G.W., M.J. Dilts, O.D. Simmons III, C.A. Likirdopulos, L.H, Krometis, M.D. Sobsey. (2005). "Microbial partitioning to settleable particles in stormwater." *Water Research*, 39, 1773 – 1782.

Crane, S. R., and J. A. Moore. (1986). "Modeling enteric bacterial die-off: a review." *Water, Air, and Soil Pollution*, 27, 411-439.

Elder, J.F. (1987). "Indicator bacteria concentrations as affected by hydrological variables in the Apalachicola River, Florida." *Water, Air, and Soil Pollution*, 32, 407-416.

Eleria, A., and R. M. Vogel. (2005). "Predicting fecal coliform bacteria levels in the Charles River, Massachusetts, USA." *Journal of the American Water Resources Association*, 41(5): 1195-1209.

Ferguson, C.M., B.G. Coote, N.J. Ashbolt, and I.M. Stevenson. “Relationships between indicators, pathogens, and water quality in an estuarine system.” Water Research, 30(9): 2045-2054.

Ferguson, C., A.M. de Roda Husman, N. Altavilla, D. Deere, and N. Ashbolt. (2003). “Fate and transport of surface water pathogens in watersheds.” *Critical Reviews in Environmental Science and Technology*, 33(3), 299 – 361.

Fries, J.S., G.W. Characklis, and R.T. Noble. (2006). “Attachment of fecal indicator bacteria to particles in the Neuse River Estuary, N.C.” *Journal of Environmental Engineering*, 132(10), 1338 – 1345.

Haile, R.W., J. Witte, M. Gold, R. Cressey, C. McGee, R.C. Millikan, A. Glasser, N. Harawa, C. Ervin, P. Harmon, J. Harper, J. Dermand, J. Alamillo, K. Barrett, M. Nides, and G. Wang. (1999). “The health effects of swimming in ocean water contaminated by storm drain runoff.” *Epidemiology*, 10(4): 355-363.

Hathaway, J.M., W.F. Hunt, and S. Jadlocki. (2009). “Indicator Bacteria Removal in Storm-Water Best Management Practices in Charlotte, North Carolina.” *Journal of Environmental Engineering*, 135(12): 1275-1285.

Hollander, M., and D.A. Wolfe. (1999). *Nonparametric Statistical Methods*, 2nd Ed., John Wiley and Sons, Inc., New York, NY.

Jan, C., C. Chang, and M. Lee. (2006). “Discussion of ‘design and calibration of a compound sharp-crested weir’ by J. Martinez, J. Reca, M.T. Morillas, and J.G. Lopez.” Journal of Hydraulic Engineering, 132(8): 868-871.

Kelsey, H., D.E. Porter, G. Scott, M. Neet, and D. White. (2004). “Using geographic information systems and regression analysis to evaluate relationships between land use and fecal coliform bacterial pollution.” *Journal of Experimental Marine Biology and Ecology*, 298: 197-209.

Kibbey, H.J., C. Hagedorn, and E.L. McCoy. (1978). “Use of fecal streptococci as indicators of pollution in soil.” *Applied and Environmental Microbiology*, 35(4): 711-717.

Krometis, L.A., G.W. Characklis, O.D. Simmons III, M.J. Dilts, C.A. Likirdopulos, and M.D. Sobsey. (2007). “Intra-storm variability in microbial partitioning and microbial loading rates.” *Water Research*, 41(2), 506 – 516.

Line, D.E., N.M. White, W.W. Kirby-Smith, and J.D. Potts. (2008). “Fecal coliform export from four coastal North Carolina areas.” Journal of the American Water Resources Association, 44(3): 606-617.

Mallin, M.A., K.E. Williams, E.C. Esham, and R.P. Lowe. (2000). “Effect of human development on bacteriological water quality in coastal watersheds.” *Ecological Applications*, 10(4): 1047-1056.

McCarthy, D.T., V.G. Mitchell, A. Deletic, and C. Diaper. (2007). "*Escherichia coli* in urban stormwater: explaining their variability." *Water Science and Technology*, 56(11), 27-34.

McCarthy, D.T. (2008). "Modelling microorganisms in urban stormwater." Doctoral dissertation, Monash University, Melbourne, Australia.

McCarthy, D.T., A. Deletic, V.G. Mitchell, T.D. Fletcher, C. Diaper. (2008b). "Uncertainties in stormwater *E. coli* levels."Water Research, 42: 1812-1824.

North Carolina Department of Natural Resources (NCDENR). (2009). "Shellfish sanitation and recreational water quality – shellfish sanitation program." http://www.deh.enr.state.nc.us/shellfish/shellfish.htm (Nov. 14, 2009).

National Oceanic and Atmospheric Administration (NOAA). (2009). "Vapor pressure calculator." http://www.srh.noaa.gov/epz/?n=wxcalc_vaporpressure (Nov. 18, 2009).

Ortega, C., H.M. Solo-Gabriele, A. Abdelzaher, M. Wright, Y. Deng, L.M. Stark. (2009). "Correlations between microbial indicators, pathogens, and environmental factors in a subtropical estuary." *Marine Pollution Bulletin*, *in press*.

Ott, R.L., and M. Longnecker. (2001). *An introduction to statistical methods and data analysis*, 5th Ed., Duxbury, Pacific Grove, CA.

SAS Institute Inc. 2001. The SAS system for Windows (Release 9.1), Cary, NC.

Schoonover, J.E. and B.G. Lockaby. (2006). "Land cover impacts on stream nutrients and fecal coliform in the lower piedmont of West Georgia." *Journal of Hydrology*, 331:371-382.

Selvakumar, A., and M. Borst. (2006). "Variation of microorganism concentrations in urban stormwater runoff with land use and seasons." *Journal of Water and Health*, 4(1): 109-124.

State Climate Office of North Carolina (SCONC). (2009). "1971 – 2000 Climate Normals." http://www.nc-climate.ncsu.edu/cronos/normals.php?station=317079 (Nov. 18, 2009).

Stevik, T. K., K. Aa, G. Ausland, and J.F. Hanssen. (2004). "Retention and removal of pathogenic bacteria in wastewater percolating through porous media: a review." Water Research, 38: 1355-1367.

Tiefenthaler, L.L., E.D. Stein, and G.S. Lyon. (2009). "Fecal indicator bacteria (FIB) levels during dry weather from Southern California references streams." *Environmental Monitoring and Assessment*, 155: 477-492.

USEPA. (1976). "Quality criteria for water." *EPA-440/9-76-023*, Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA). (1983). “Methods for Chemical Analysis of Water and Wastes.” *EPA 600/4-79/020*. Office of Research and Development, Washington, DC.

USEPA. (1986). “Ambient water quality criteria for bacteria – 1986.” *EPA 440/5-84-002*. Office of Water, Washington, DC.

USEPA. (2001). “Protocol for developing pathogen TMDLs.” *EPA-841-R-00-002*. Office of Water, Washington, DC.

USEPA. (2002). “Urban stormwater BMP performance monitoring.” *EPA-821-B-02-001*. Office of Water, Washington, DC.

USEPA. (2003). “Bacterial water quality standards for recreational waters: status report.” *EPA-823-R-03-008*. Office of Water, Washington, DC.

Van Donsel, D.J., E.E. Geldreich, and N.A. Clarke. (1967). “Seasonal variations in survival of indicator bacteria in soil and their contribution to storm-water pollution.” Applied Microbiology, 15(6): 1362-1370.

Wang, L., K.R. Mankin, and G.L. Marchin. (2004_. “Survival of fecal bacteria in dairy cow manure.” *Transactions of the ASAE*, 47(4): 1239-1246.

Yacub, G.P., D.A. Castric, K.L. Stadterman-Knauer, M.J. Tobin, M. Blazina, T.N. Heineman, G.Y. Yee, L. Frazier. (2002). “Evaluation of Colilert and Enterolert defined substrate methodology for wastewater applications.” *Water Environment Research*, 74(2), 131 – 135.

Young, K.D., and E.L. Thackston. (1999). “Housing density and bacterial loading in urban streams.” *Journal of Environmental Engineering*, 125: 1177-1180.

3 Evaluation of First Flush for Indicator Bacteria and Total Suspended Solids in Urban Stormwater Runoff

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3.1 Abstract

An urban watershed in Raleigh, NC, was evaluated for *Escherichia coli (E. coli)*, fecal coliform, enterococci, and Total Suspended Solids (TSS) over 20 storm events. Sampling procedures allowed collection of multiple discrete samples per event, resulting in a relatively detailed description of mass export for each storm. Data were evaluated to determine if a first flush effect was present for indicator bacteria and TSS in stormwater runoff. Analyses suggested there was a significant first flush effect for fecal coliform and TSS, although the first flush effect for fecal coliform was relatively weak. For *E. coli* and enterococci, no significant first flush effect was noted. Generally, the first flush effect was not always present for indicator bacteria and, if present, tended to be weak. The first flush effect for TSS was substantially stronger than that of any indicator bacteria. Further analysis showed poor correlation between first flush strength and antecedent climate variables, storm characteristics, and flow characteristics. However, seasonal differences for first flush strength were noted. Specifically, winter storms showed a stronger first flush effect for all indicator bacteria. The results of this study indicate that stormwater runoff presents a public health hazard due to elevated indicator bacteria levels for all portions of the storm event. Further, stormwater management practices cannot be expected to treat proportionally more indicator bacteria when sized for the water quality event. Instead, removal will simply be a function of a management practice's volume capture and microbe sequestration efficiency.

3.2 Introduction

In the United States Environmental Protection Agency's (USEPA) National Water Quality Inventory in 2006, 12% of stream and river miles were impaired by indicator bacteria (USEPA 2008). Stormwater

runoff has been identified as a contributor to indicator bacteria in surface waters. However, despite concerns over water quality degradation due to indicator bacteria in stormwater runoff, numerous facets of microbial transport and fate are poorly understood.

Many pollutants in stormwater runoff are commonly thought to exhibit a “first flush” transport pattern. Essentially, that a larger proportion of pollutant mass or higher pollutant concentrations are expected during the initial stages of a storm event (Sansalone and Cristina 2004). First flush patterns have been evaluated in urban stormwater runoff for multiple pollutants including sediments, oil and grease, metals, nutrients, chemical oxygen demand, pH, temperature, and conductivity (Barrett et al. 1998, Bertrand-Krajewski et al. 1998, Characklis and Wiesner 1997, Deletic 1998, Flint and Davis 2007, Lee et al. 2002, Sansalone and Buchberger 1997, Sansalone and Cristina 2004, Stenstrom et al. 1984). However, first flush patterns have not been consistently noted in urban watersheds and may depend on such factors as storm size, rainfall intensity, watershed characteristics, and various hydrologic and transport factors (Deletic 1998, Sansalone and Cristina 2004).

Various methodologies have been employed to evaluate the first flush effect. In a review by Sansalone and Cristina (2004), first flush analyses were placed into three categories based on the approach taken by the researchers: mass based, concentration based, and empirically based. For detailed first flush analyses, mass based procedures have been commonly used. Within the mass based procedure, there are multiple methodologies which have been used to evaluate the first flush; however, Sansalone and Cristina (2004) showed similar conclusions would be made when each of the methods was applied to a common experimental data set.

Despite numerous studies characterizing the first flush for pollutants in urban stormwater, relatively few detailed studies have been performed for indicator bacteria, particularly *Escherichia coli* (*E. coli*) and enterococci. *E. coli* and enterococci are commonly used to regulate microbial water quality in the United States, Europe, Australia, New Zealand, and elsewhere. A report by the California Department of Transportation (2000) determined a first flush was not visible for fecal coliform in highway runoff during eight storms at two locations. However, the first flush was evaluated based primarily on qualitative analysis of pollutographs. Similar conclusions resulted from an analysis of

fecal coliform and fecal streptococcus data taken from the National Stormwater Quality Database (Maestre and Pitt 2004). Non-parametric statistical comparisons were made between samples taken within the first 30 minutes of a storm event and composite samples for the same event. No statistical differences in concentrations were found. Other studies by Krometis et al. (2007) showed decreased concentrations of *E. coli* and fecal coliform in the latter portions of storm flow in streams, indicating a potential first flush effect. Conversely, enterococci were found to remain relatively consistent throughout the storm. Krometis et al. (2007) concluded that the greatest percentage of settlable microbes was exported in the first 50% of runoff volume. However, indicator bacteria transport processes in streams may differ from those in urban stormwater systems.

Recent studies by McCarthy (2009) provided detailed analysis of the first flush for *E. coli* in four urban watersheds in Melbourne, Australia. McCarthy (2009) showed a consistent first flush was not present for any of the four watersheds; however, a first flush effect was statistically found in the medium density residential watershed. Further, McCarthy (2009) tested associations between the first flush strength and antecedent climate parameters, storm characteristics, and flow characteristics. No variable was identified which could consistently explain variations in the first flush strength for all sites. It should be noted that the weather patterns in Melbourne, Australia, differ from those in the Southeastern United States, potentially leading to differences in microbial behavior. Differences in weather include higher average yearly rainfall, higher average summer temperatures, and lower average winter temperatures in Raleigh, NC (ABOM 2009, SCONC 2009).

Such evaluations are important, as Best Management Practices (BMPs) are designed to treat the runoff associated with a pre-determined water quality rainfall depth. To facilitate efficient use of land and monetary resources, this depth is often selected under the perception that a first flush exists. Subsequently, capture and treatment of the initial portion of the storm is believed to result in maximum pollutant capture relative to runoff volume capture. Determining if a first flush exists for indicator bacteria is important in determining the efficiency of stormwater BMPs for treatment of microbes. Stormwater BMPs are also known as Sustainable Urban Drainage Systems (SUDS) and Water Sensitive Urban Designs (WSUDs).

Further, understanding indicator bacteria transport patterns may result in better decisions regarding public health. The presence of a first flush would suggest high concentrations of indicator bacteria may reach recreational waters quickly after a storm begins. Further, variations in indicator bacteria concentrations during storm events have important ramifications for monitoring, particularly with the common use of grab samples for indicator bacteria.

The objectives of this study were to build upon the current understanding of microbial processes by: (1) evaluating the presence of a first flush in urban stormwater for multiple indicator bacteria, all of which are used in some capacity in the United States and elsewhere, (2) examining correlating factors between first flush strength and antecedent climate conditions to see if relationships differ for the humid, warm, Southeastern United States, (3) comparing results for indicator bacteria to those of Total Suspended Solids (TSS), which has been shown to exhibit a first flush effect and includes particles on which microbes are known to attach.

3.3 Materials and Methods

3.3.1 Site Description

Data were collected from a 5.1-ha watershed in Raleigh, NC, with approximately 35% total imperviousness (Figure 3.1). Connected impervious area accounted for 15% of the watershed and consisted primarily of roadways. The watershed was developed prior to USEPA stormwater management regulations, and thus contained no stormwater management practices. The watershed contained separate stormwater and wastewater sewer systems. Sewer cross-connection was not expected as the stormwater outfall for the watershed was noted to be completely dry on multiple occasions during the late summer/early fall. However, groundwater intrusion was likely as base flow was noted during other periods of the year.

3.3.2 Monitoring Methods

A compound weir was installed in a 76-cm reinforced concrete pipe which served as the outlet for the watershed. Depth in the pipe was recorded using an ISCO 730 bubbler module. A stage-discharge relationship developed for triangular-rectangular compound weirs by Jan et al. (2006) was

used to estimate flow from depth readings. The bubbler module was attached to an ISCO Avalanche refrigerated sampler. A multi-bottle arrangement was used in the sampler. Fourteen polypropylene bottles were utilized in the configuration. Samples and level readings were collected from the invert of the stormwater pipe. McCarthy et al. (2008) indicated this collection point did not yield significantly different results than a collection point at the top of the water column for indicator bacteria.

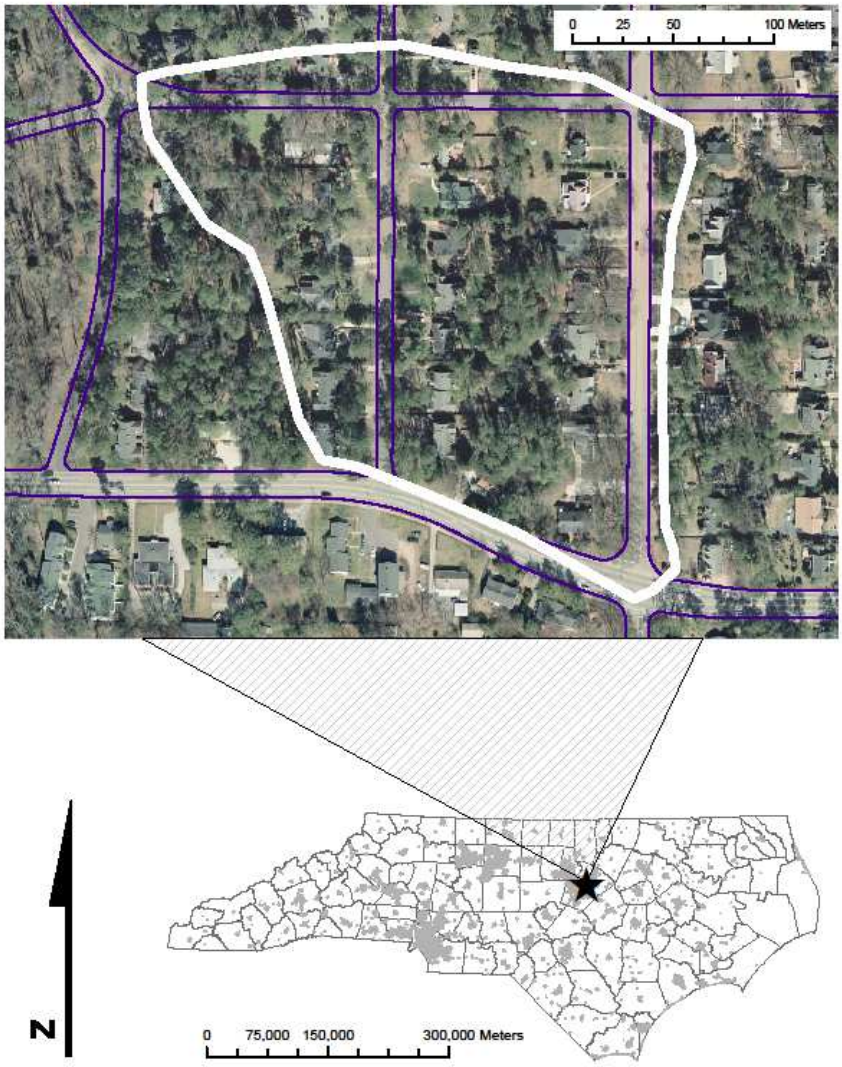


Figure 3.1: Ariel view of watershed (boundary in white) and study location in North Carolina, USA (★)

All tubing and bottles were washed, rinsed with deionized water, and autoclaved at 121^{o}C for 20 minutes prior to each anticipated storm to maintain sterility. The multi-bottle configuration was used to collect discrete, flow paced samples. Flow pacing was adjusted prior to and during storms to allow collection of an adequate number of samples. If adjustments were needed during a storm, flow pacing was increased later in the storm when storm flow and bacteria concentrations were assumed to be less variable. Stormwater samples were transported to the Department of Biological and Agricultural Engineering at North Carolina State University and stored under refrigeration until analyzed.

A tipping bucket rain gage was installed just outside the experimental watershed in a location with an appropriate lack of tree canopy. A HOBO data logger recorded data from the tipping bucket, which was validated using an on-site manual rain gage. Watershed and monitoring characteristics are summarized in Table 3.1.

Table 3.1: Summary of watershed and monitoring specifications

Watershed and Monitoring Specifications	
Location	35.80^{o}N, 78.67^{o}W
Watershed Area	5.1 ha
Total Imperviousness	35%
Connected Imperviousness	15%
Description	Medium density residential with mature tree canopy
Soil Type (WCCOR 2010)	Cecil - sandy loam
Monitoring Primary Device	compound weir
Distance of rain gage to furthest extent of watershed	560 m

Climate data from the Lake Wheeler Road Field Laboratory, located approximately 8.3 km from the experimental watershed, were utilized for additional analyses. Data were averaged as appropriate to establish antecedent climate conditions over a range of time periods (previous 1, 2, 7, 14, and 28 days). Variables used for the analysis were air temperature, vapor pressure, relative humidity, potential evaporation, solar radiation, and total rainfall. Vapor pressure was calculated from

temperature using standard equations (NOAA 2009). Historical average temperature and precipitation data for Raleigh, NC, are presented in Table 3.2.

Table 3.2: Historical climate data for Raleigh, NC (SCONC 2009)

Parameter	Jan	Feb	Mar	Apr	May	June	Jul	Aug	Sept	Oct	Nov	Dec	Annual
Average Total Rainfall (cm)	11.3	9.0	11.3	7.6	10.2	10.3	11.0	10.9	10.8	9.6	7.8	8.2	117.9
Average Daily Maximum Temperature (^{o}C)	9.3	11.7	16.2	21.4	25.3	29.1	31.1	29.9	26.7	21.0	16.3	11.2	20.8
Average Daily Minimum Temperature (^{o}C)	-1.1	0.2	4.3	8.8	13.7	18.3	20.8	20.1	16.6	9.7	5.3	0.8	9.8

3.3.3 Sample Analysis

Indicator bacteria analyses were generally performed within 24 hours of sample collection. Samples required either a 100:1 or 1000:1 dilution due to high bacteria counts. The Colilert method (defined substrate technologies; IDEXX, Westbrook, Maine) was modified to detect fecal coliform and *E. coli.* Samples were incubated at 37^{o}C for 1 to 3 hours followed by incubation at 44.5^{o}C for 21 to 23 hours (Yakub et al. 2002). Enterococci were enumerated using Enterolert (defined substrate technologies; IDEXX, Westbrook, Maine), which was performed per manufacturer guidelines. Positive and negative controls were used to validate laboratory analyses, but enterococci standards were not used until the latter two thirds of the study due to a lack of supply. The remainder of each discrete sample was tested for TSS using SM 2540D (APHA, AWWA, and WEF 1998) at the North Carolina Center for Applied Aquatic Ecology (NCCAAE).

3.3.4 Data Analysis

Discrete, flow paced samples were collected during the course of each storm event. The concentrations of TSS and indicator bacteria in each sample were compiled with flow data collected at two minute intervals to calculate pollutant mass export for each sampling interval. These data

were then used to generate dimensionless relationships for volume and mass for each storm event. Cumulative volume and mass were calculated for each storm at each sampling time (t_k). These cumulative values were then normalized by the total mass or volume of the storm (Equations 1 and 2).

$$v(t_k) = \frac{\sum_{i=0}^{i=k} Q_i \times t_i}{\sum_{i=0}^{i=t} Q_i \times t_i} \qquad (1)$$

$$m(t_k) = \frac{\sum_{i=0}^{i=k} Q_i \times t_i \times C_i}{\sum_{i=0}^{i=t} Q_i \times t_i \times C_i} \qquad (2)$$

In equations 1 and 2, $v(t_k)$ and $m(t_k)$ are the cumulative volume or mass at any time t_k normalized by the total volume or mass for a given event. Each pair of normalized values ($v(t_k)$ and $m(t_k)$) were plotted for a given storm event to evaluate the presence of a first flush. The first flush was indicated by the plot of normalized values lying above a 45^o line, thus signifying the largest proportion of mass left the watershed in the initial portion of the rain event (Figure 3.2). However, the first flush is typically evaluated at some percentage of the total runoff volume, whereby the total mass exported at this percent volume is compared to a chosen threshold value. The percent of total volume used for evaluation varies. Sansalone and Cristina (2004) and Deletic (1998) evaluated the first flush at 20% of the total storm volume, Flint and Davis (2007) at 25%, and Bertrand-Krajewski et al. (1998) suggested a value of 30%. To provide comparison to similar studies, 30% will be used, similar to that of McCarthy (2009). Essentially, the proportion of total mass transported during the first 30% of the total storm volume was determined and labeled FF_{30} (as in McCarthy 2009), with a first flush effect being noted if FF_{30} is greater than 30% (Figure 3.2). The strength of the first flush effect was also evaluated based on the magnitude of the FF_{30}.

Other criterion have been used which rely on exact thresholds to verify a first flush effect. Thus, data will also be evaluated via a threshold used by Wanielista and Yousef (1993) and Flint and Davis (2007). Both authors selected a criterion whereby 50% of the total mass must be transported in the first 25% of runoff for a storm to be defined as truly exhibiting a first flush.

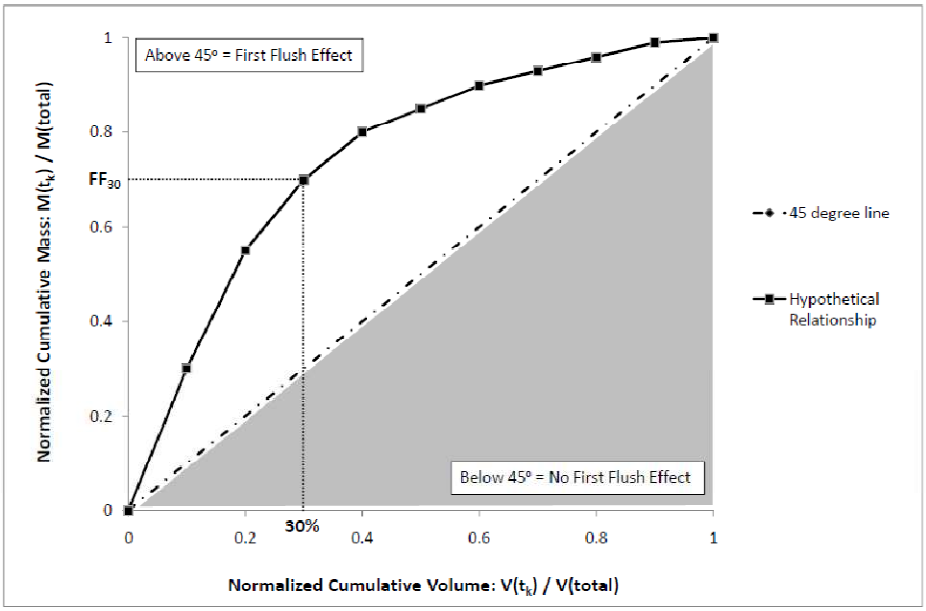


Figure 3.2: Illustration of data analysis method

3.4 Results and Discussion

Overall, 20 storms were evaluated for indicator bacteria between October 2008 and September 2009. Analysis for TSS began in February 2009 and continued for 13 events. An average of 10 discrete samples were collected for each storm, with no storm having fewer than 5 discrete samples. Event mean concentrations were developed by Hathaway and Hunt (in review) and are summarized in Table 3.3.

Table 3.3: Summary statistics for collected data

Statistic	Rain (cm)	Event Mean Concentrations (EMCs)			
		E. coli (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)	TSS (mg/L)
Geometric Mean	1.59	15,396	43,148	11,475	114
Mean	1.96	25,643	80,142	25,155	140
Median	1.75	15,010	59,442	12,342	122
Standard Deviation	1.25	24,323	82,931	40,380	90
Max	5.59	84,688	342,405	181,846	309
Min	0.41	710	1,469	1,306	33

3.4.1 Analysis of First Flush Effect – General Observations

Plots of normalized flow vs. normalized mass for TSS and each indicator bacteria are presented in Figures 3.3a – 3.3d. Plots showed fairly even distribution of storms on either side of the 45^{o} line for *E. coli* and enterococci. On average, fecal coliform appeared to be slightly distributed above the 45^{o} line. TSS appeared to have a substantially stronger first flush effect than the indicator bacteria with few storms lying below the 45^{o} line. Most storms for indicator bacteria were nearly as likely to be below the 45^{o} line as above.

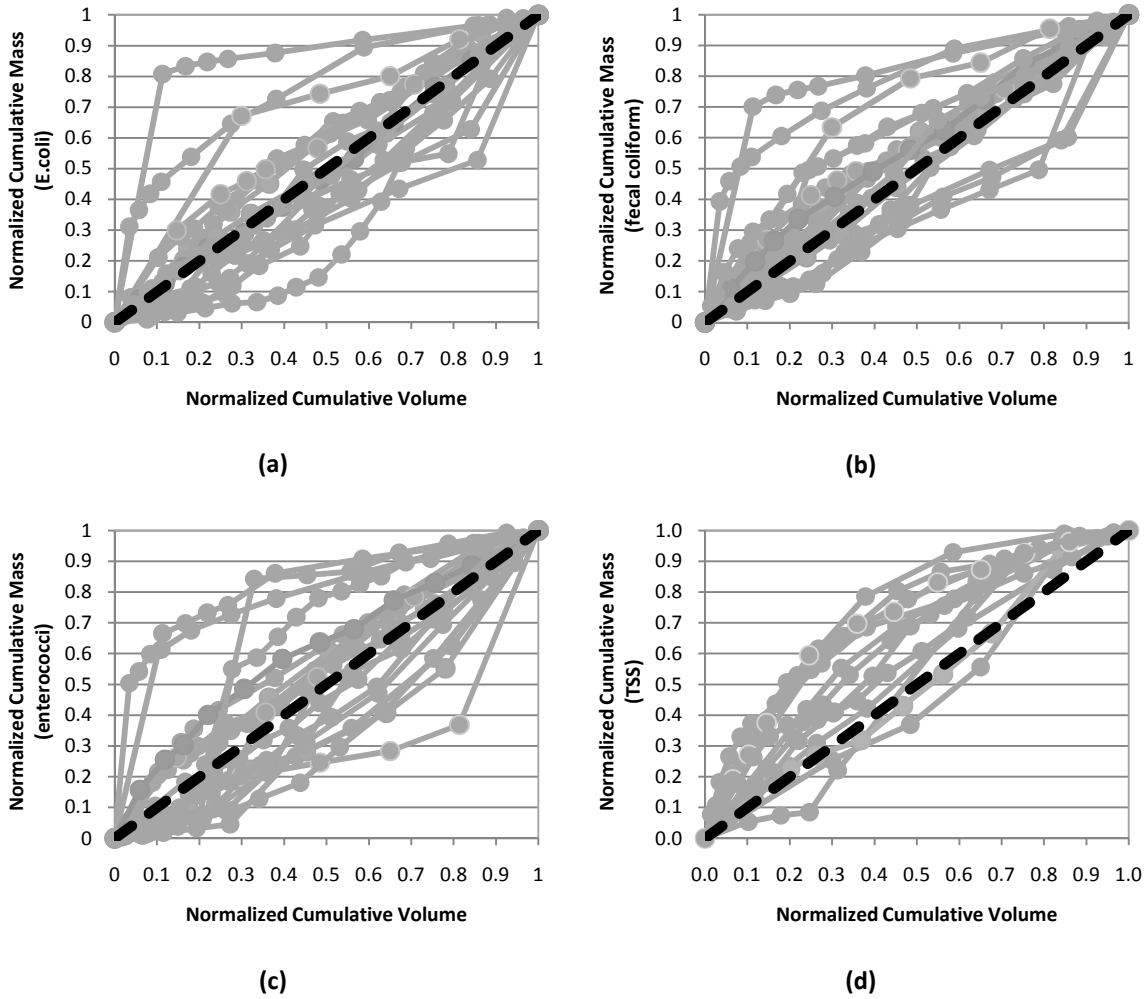


Figure 3.3: Normalized volume vs. normalized mass for (a) *E. coli*, (b) fecal coliform, (c) enterococci, and (d) TSS

During some storm events, the final portion of runoff had the largest indicator bacteria concentrations, described by McCarthy (2009) as an "end flush." McCarthy (2009) attributed this end flush to possible wastewater intrusion into the stormwater pipe. However, it is also possible that the land use contributing to stormwater runoff near the end of the storm event, likely pervious areas, had high concentrations of indicator bacteria. In this experimental watershed, such pervious areas were frequently residential yards where domestic animals were common.

3.4.2 Analysis of First Flush Effect – FF30

The FF_{30} was determined for each pollutant and compiled in Table 3.4. The FF_{30} can describe the strength of the first flush effect for a given storm event. As FF_{30} increases, the percentage of total mass delivered in the first 30% of runoff increases. The first flush effect was not evident for any pollutant for all storm events. However, some degree of first flush effect was determined for *E. coli* during 45% of events, for enterococci during 50% of events, for fecal coliform during 70% of events , and for TSS during 77% of events.

On average, the FF_{30} for *E. coli* was 35% with a maximum of 86%. Enterococci also had an average FF_{30} of 35%, with a max of 79%. Fecal coliform was slightly higher with an average FF_{30} of 39% and high of 78%. TSS had the highest average FF_{30} at 46%, with a maximum of 67%. Wilcoxon Signed Rank analyses showed that *E. coli* and enterococci did not exhibit a median FF_{30} significantly different than 30% ($p < 0.05$). The median FF_{30} for fecal coliform was significantly different than 30% ($p = 0.047$), as was the median FF_{30} for TSS ($p = 0.009$). Further statistical analysis indicated that although the first flush effect was stronger for some pollutants, no statistical differences in FF_{30} could be found for any of the pollutants (Table 3.5). This is likely due to the high amount of variability in FF_{30} that was noted for each pollutant, as evidenced by the standard deviations in Table 3.4.

Table 3.4: FF_{30} for collected data

Date	FF_{30} (as %)			
	E. coli	**enterococci**	**fecal coliform**	**TSS**
10/17/2008	6	56	36	-
11/4/2008	41	27	31	-
11/14/2008	25	26	27	-
11/25/2008	18	9	35	-
12/20/2008	23	20	45	-
1/6/2009	39	38	40	-
1/28/2009	43	46	53	-
2/11/2009	86	79	78	67
2/18/2009	42	39	37	34
3/13/2009	66	74	71	60
3/26/2009	18	36	19	19
4/2/2009	67	20	63	25
5/8/2009	14	14	16	29
5/14/2009	45	37	45	62
6/4/2009	29	48	40	41
7/17/2009	27	67	20	62
7/25/2009	33	19	27	52
8/5/2009	14	10	19	47
8/28/2009	29	16	32	64
9/7/2009	28	21	53	40
mean =	35	35	39	46
median =	29	31	37	47
st dev =	20	21	17	16
minimum =	6	9	16	19
maximum =	86	79	78	67

Table 3.5: Wilcoxon signed-rank analysis of differences in FF_{30} (p-values)

Pollutant	**fecal coliform**	**enterococci**	**TSS**
E. coli	0.165	0.5217	0.2163
fecal coliform	-	0.2162	0.3396
enterococci	-	-	0.1909

These results are similar to first flush evaluations on *E. coli* for four watersheds in Melbourne, Australia, by McCarthy (2009). McCarthy (2009) observed that none of the watersheds consistently exhibited a first flush for *E. coli*. However, a significant first flush effect was identified for a medium density residential watershed ($p < 0.05$). Average FF_{30} for *E. coli* for the four watersheds studied by McCarthy (2009) was between 30 and 40%, similar to the results of this analysis.

Conversely, sediments have been shown to exhibit some degree of first flush effect in such studies as Flint and Davis (2007) who showed 70% of storms exhibited flushing for TSS, Bertrand-Krajewski et al. (1998) where 80% of storms from watersheds with separate sewer systems had normalized mass vs. normalized volume curves above the 45^{o} line for TSS, Sansalone and Cristina (2004) who showed the percent of total mass of both dissolved solids and suspended sediment concentrations was higher than percent of total volume at the threshold of 20%, and Deletic (1998) where the first 20% of runoff carried 25.5% and 30.8% of suspended solids for two watersheds studied. It should be noted that the methodologies employed by these studies varied and that conclusions as to whether a first flush effect was exhibited were based on varying thresholds. Thus, conclusions for each study may differ from this analysis, where a first flush is identified simply by the percent total mass being larger than the percent total volume during the beginning of the runoff event.

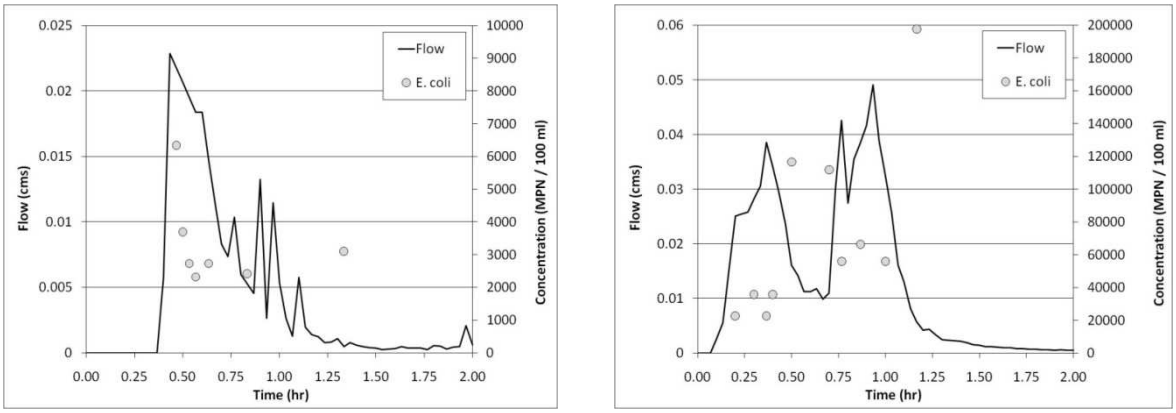
Despite variations in methodologies, the results of this study and others in scientific literature imply that sediments can exhibit a first flush effect. Thus, TSS can be considered a type of control which shows that the first flush effect *is possible* given the hydrologic regime in this watershed. However, *E. coli* and enterococci did not follow this pattern. This indicates the potential for different sources and/or transport mechanisms for TSS and indicator bacteria in urban watersheds. It should be noted that fecal coliform exhibited a stronger first flush effect that other indicator bacteria.

Differences in TSS and indicator bacteria transport were further explored by performing correlation analyses on TSS and indicator bacteria for each discrete sample taken during a given storm event. Spearman correlation coefficients generated for each storm event were averaged. This allowed further description of intra-event relationships between TSS and indicator bacteria. TSS was poorly correlated to indicator bacteria with average spearman coefficients of 0.08, 0.10, and 0.01 for TSS –

E. coli, TSS – fecal coliform, and TSS – enterococci, respectively. This further supports the assertion that TSS and indicator bacteria may have different sources and/or transport patterns in urban stormwater runoff, and that high concentrations of TSS do not necessarily correspond to high concentrations of indicator bacteria. Although indicator bacteria can attach to and travel with particles (Characklis et al. 2005, Krometis et al. 2007), complicating factors likely make relationships between TSS and indicator bacteria hard to identify, particularly considering the large range of particle sizes represented by TSS measurements. Such complicating factors include differences in attachment based on particle size (Davies and Bavor 2000), differences in sorption based on soil type (Mankin et al. 2007), and natural variability /analytical uncertainty for both indicator bacteria and TSS (Characklis et al. 2005).

3.4.3 Analysis of First Flush Effect – Threshold Methodology

Numerous studies have applied a threshold methodology to determine if a given plot of cumulative volume to cumulative mass constitutes a first flush (Deletic 1998, Sansalone and Cristina 2004, Bertrand-krajewski et al. 1998, Wanielista and Yousef 1993, Flint and Davis 2007). For this analysis, the threshold was taken to be 50% of the total mass being transported in the first 25% of runoff (similar to Flint and Davis 2007). Total mass was rounded to the nearest one percent. TSS had the greatest number of events exhibiting a first flush effect with 5 of 13 events, or 38%, having greater than 50% of the total mass transported in the first 25% of runoff. This was higher than that reported by Flint and Davis (2007), where only 17% of storms exhibited a first flush. A Wilcoxon signed rank analysis showed that TSS cumulative mass was not significantly different than 50% for the storms monitored ($p = 0.057$). Fecal coliform reached the first flush threshold on 5 of 20 events (25%). Cumulative mass was significantly lower than 50% ($p = 0.002$). *E. coli* and enterococci had the least number of storms meeting the first flush threshold with 3 of 20 storms and 2 of 20 storms, respectively. Cumulative mass for both *E. coli* and enterococci was significantly less than 50% ($p = 0.006$ and $p = 0.004$, respectively). Figure 3.4 shows example data from storm events which exhibited (Figure 3.4a) and did not exhibit (Figure 3.4b) a first flush effect for *E. coli*.



(a) (b)

Figure 3.4: Flow vs. *E. coli* concentration for (a) 2/11/2008 – first flush effect evident and (b) 5/18/2009 – no first flush effect evident

3.4.4 Correlation analysis

Antecedent climate, storm, and runoff variables were correlated to FF_{30} for each indicator bacteria and TSS (Table 3.6). Resultant Spearman ranks are presented in Table 3.7 for relationships which were significant at $\alpha = 0.05$. Few parameters were correlated to FF_{30} for *E. coli* and fecal coliform. Further, no parameters were correlated to enterococci and TSS. Similarly, few antecedent climate, storm, and flow parameters were found to be correlated to *E. coli* for 3 of 4 watersheds studied by McCarthy (2009). Relationships impacting the build-up and wash-off of indicator bacteria are complex, making correlation analyses difficult. Indicator bacteria transport and fate in urban watersheds is likely influenced by such factors as climate, soil properties, interactions between microbes, land use, and dynamics within stormwater conveyances (Crane and Moore 1985, Haydon and Deletic 2006, McCarthy 2008b)

Parameters were generally negatively correlated to *E. coli* and fecal coliform and were either temperature itself or associated with temperature (in the Southeast United States), such as relative humidity and vapor pressure. Analysis by Hathaway and Hunt (in review) found that as temperature increased in the watershed, indicator bacteria EMCs increased. Thus, it is possible that widespread abundance of indicator bacteria during warmer temperatures results in a lack of first flush, as the

supply of bacteria is not limited and not concentrated in a particular part of the watershed or stormwater conveyance system.

Table 3.6: Variables used in correlation analysis

Variable	Type
Flow duration	runoff
Average flow rate	runoff
Peak flow rate	runoff
Total runoff volume	runoff
Total rainfall	rainfall
Storm duration	rainfall
Antecedent dry period	rainfall
Antecedent period since 0.5 cm of rainfall	rainfall
Max 5 minute intensity	rainfall
Average intensity	rainfall
Air temperature	climate*
Relative humidity	climate
Vapor pressure	climate
Solar radiation	climate
Total rainfall	climate
Potential evapotranspiration	climate

*climate variables averaged over antecedent 1, 2, 7, 14, and 28 days

Table 3.7: FF_{30} correlation analysis

Variable	*E. coli*		fecal coliform		Enterococci		TSS	
	ρ	p-value	ρ	p-value	ρ	p-value	ρ	p-value
enterococci	-	-	0.48	0.0323	-	-	-	-
Air Temperature 14 days	-	-	-0.46	0.039	-	-	-	-
Air Temperature 28 days			-0.50	0.0245	-	-	-	-
Relative Humidity 14 days	-0.54	0.0137			-	-	-	-
Vapor Pressure 28 days			-0.46	0.039	-	-	-	-

Significant correlations were also noted between fecal coliform and enterococci FF_{30} (correlation coefficient = 0.48, p = 0.032). *E. coli* and enterococci had a spearman correlation coefficient of 0.44,

but the relationship was not significant (p = 0.054). These data indicate that first flush strength is somewhat linear among indicator bacteria. Thus, although average FF_{30} may vary, factors influencing the FF_{30} may be reasonably similar among indicator bacteria types. *E. coli* and fecal coliform were not tested for correlation due to concerns over independence of the data given the analytical methodology.

3.4.5 Investigation of Seasonal Differences

Based on the results of the correlation analysis, further examination was performed to determine the affect of season on FF_{30}. Mean seasonal FF_{30} for each indicator bacteria is presented in Figure 3.5. Kruskal-Wallis analyses were performed to determine if seasonality significantly influenced FF_{30}. This was followed by Wilcoxon Rank Sum analyses for pairwise comparisons. The results of these analyses are presented in Table 3.8.

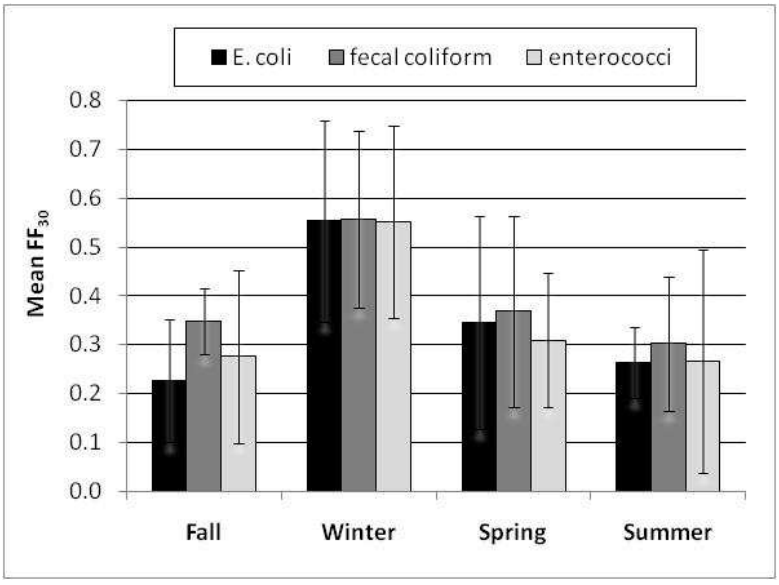


Figure 3.5: Mean seasonal FF_{30} for indicator bacteria (with standard deviation)

Table 3.8: Statistical analysis of seasonal differences in FF_{30} (p-values)

Indicator Bacteria	Kruskal-Wallis	Wilcoxon Rank Sum					
		fall - winter	fall - spring	fall - summer	winter - spring	winter - summer	spring - summer
E. coli	***0.0479***	***0.0216***	0.4633	0.4034	***0.0122***	0.2963	0.6742
fecal coliform	0.1202	***0.0367***	0.9166	0.3457	0.2963	***0.0122***	0.6742
enterococci	0.0868	0.0601	0.7533	0.6761	0.0601	0.0601	0.5309

Significant Kruskal-Wallis values were only noted for *E. coli*; however, pairwise comparisons showed significant differences among seasons for both *E. coli* and fecal coliform ($p < 0.05$). Significant differences were generally observed in comparisons involving the winter season. Seasonal relationships for enterococci were not significant, but low p-values ($p = 0.06$) were noted in all analyses comparing winter. Mean seasonal FF_{30} for the winter is the highest among all seasons. No winter storm event for any of the three indicator bacteria had an FF_{30} less than 30%.

Winter indicator bacteria concentrations were shown to be lowest by Hathaway and Hunt (in review) and Selvakumar and Borst (2006). Thus, indicator bacteria during the winter may be source limited. McCarthy (2009) surmised that indicator bacteria persisting within stormwater pipes may be washed out during some rain events, giving the appearance of a first flush. It is possible this phenomenon combined with reduced sources during the winter, from such factors as diminished wild and domestic animal activity, may produce a first flush effect as concentrations lessen through the storm event. These processes are not well understood and further study is needed to verify these postulations.

3.5 Conclusions

An urban watershed was monitored for 20 storm events for *E. coli*, fecal coliform, enterococci, and for 13 events for TSS. Multiple discrete samples were taken during the course of each storm event, allowing detailed analysis of mass transport from the watershed. Results show the FF_{30} for *E. coli* and enterococci is not significantly different than 30%, demonstrating no greater proportion of mass loading at the beginning of storms ($p < 0.05$). A significant first flush effect was noted for fecal

coliform and TSS ($p<0.05$), TSS having the most pronounced first flush effect. Further analysis suggested FF_{30} was not significantly different among any of the pollutants, emphasizing the variability in FF_{30} that was observed in this study. First flush strength was fairly well correlated among indicator bacteria, suggesting similar transport and fate mechanisms influence the first flush effect for microbes.

Data were also analyzed based on a threshold methodology, which contends that a true first flush effect does not exist unless a prescribed threshold is reached. For this study, a threshold of 50% of the total mass being transported in the first 25% of runoff volume was used (similar to Wanielista and Yousef 1993, Flint and Davis 2007). Based on this criterion, no pollutant showed a first flush effect more than 35% of the time. TSS met the criterion most frequently, followed by fecal coliform, *E. coli*, and enterococci.

Statistical analyses generally showed poor correlation between explanatory variables and pollutant FF_{30}. However, seasonal differences among FF_{30} were noted for indicator bacteria. Winter FF_{30} was highest for each indicator bacteria, and pairwise statistical analyses commonly identified winter as statistically different than other seasons for *E. coli* and fecal coliform.

There are numerous implications of this research related to public health and environmental management. The results of this study suggest indicator bacteria concentrations can remain high even as stormwater flow decreases during the falling limb of the hydrograph. Thus, public health risks will continue throughout the entire runoff event, even if rainfall has ceased.

These data also suggest that treating a water quality volume (e.g. that associated with a 2.5 cm storm event) may not, on average, result in treating proportionally more indicator bacteria. No additional effectiveness should be assumed due to treatment of a first flush. This is important in determining stormwater BMP functionality for watershed restoration. BMP effectiveness will be a function of volume capture and treatment efficiency. In other words, if a designer wishes to treat 90% of the microbial load on an annual basis, the BMP needs to be sized to capture 90% of annual runoff.

3.6 Acknowledgements

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3.7 References

American Public Health Association, American Water Works Association, and Water Environment Federation (APHA, AWWA, and WEF). (1998). *Standard methods for the examination of water and wastewater*. 20th edition. American Public Health Association, Alexandria, VA.

Australian Bureau of Meteorology (ABOM). (2010). Climate statistics for Australian locations: summary statistics for Melbourne regional office. http://www.bom.gov.au/climate/averages/tables/cw_086071.shtml (February 16, 2010).

Barrett, M.E., L.B. Irish Jr., J.F. Malina Jr., R.J. Charbeneau. (1998). Characterization of highway runoff in Austin, Texas, area. *Journal of Environmental Engineering*, 124(2): 131-137.

Bertrand-Krajewski, J., G. Chebbo, A. Saget. (1998). Distribution of pollutant mass vs volume in stormwater discharges and the first flush phenomenon. *Water Research*, 32(8): 2341-2356.

California Department of Transportation. (2000). First Flush Study 1999-2000 Report. Rep No. CTSW-RT-00-016, Division of Environmental Analysis, Sacramento, CA.

Characklis, G.W., and M.R. Wiesner. (1997). Particles, metals, and water quality in runoff from large urban watershed. *Journal of Environmental Engineering*, 123(8): 753-759.

Characklis, G.W., M.J. Dilts, O.D. Simmons III, C.A. Likirdopulos, L.H, Krometis, M.D. Sobsey. (2005). Microbial partitioning to settleable particles in stormwater. *Water Research*, 39, 1773 – 1782.

Crane, S. R., and J. A. Moore. (1986). Modeling enteric bacterial die-off: a review. *Water, Air, and Soil Pollution*, 27, 411-439.

Davies, C.M. and H.J. Bavor. (2000). The Fate of Stormwater-Associated Bacteria in Constructed Wetland and Water Pollution Control Pond Systems. *Journal of Applied Microbiology*. 89: 349-360.

Deletic, A. (1998). The first flush load of urban surface runoff. *Water Research*, 32(8): 2462-2470.

Haydon, S., and A. Deletic. (2006). Development of a coupled pathogen-hydrologic catchment model. *Journal of Hydrology*, 328: 467-480.

Flint, K.R., and A.P. Davis. (2007). Pollutant mass flushing characterization of highway stormwater runoff from an ultra-urban area. *Journal of Environmental Engineering*, 133(6): 616-626.

Hathaway, J.M., and W.F Hunt. (in review). Statistical evaluation of factors affecting indicator bacteria in urban stormwater runoff. *Journal of Environmental Engineering*.

Lee, J.H., K.W. Bang, L.H. Ketchum, J.S. Choe, and M. J. Yu. (2002). First flush analysis of urban storm runoff. *The Science of the Total Environment*, 293: 163-175.

Jan, C., C. Chang, and M. Lee. (2006). Discussion of 'design and calibration of a compound sharp-crested weir' by J. Martinez, J. Reca, M.T. Morillas, and J.G. Lopez. *Journal of Hydraulic Engineering*, 132(8): 868-871.

Krometis, L.A., G.W. Characklis, O.D. Simmons III, M.J. Dilts, C.A. Likirdopulos, and M.D. Sobsey. (2007). Intra-storm variability in microbial partitioning and microbial loading rates. *Water Research*, 41(2), 506 – 516.

Maestre, A., R. Pitt, and D. Williamson. (2004). Nonparametric statistical tests comparing first flush and composite samples from the national stormwater quality database. In: *Innovative Modeling of Urban Water Systems*, Vol. 12. Ed. W. James, 317-338. Guelph, Ontario: CHI.

Mankin, K.R., L. Wang, S.L. Hutchinson G.L. Marchin. (2007). *Escherichia coli* sorption to sand and silt load soil. *Transactions of the ASABE*, 50(4): 1159-1165.

McCarthy, D.T., A. Deletic, V.G. Mitchell, T.D. Fletcher, C. Diaper. (2008). Uncertainties in stormwater *E. coli* levels. *Water Research*, 42: 1812-1824.

McCarthy, D.T. (2008b). Modelling microorganisms in urban stormwater. Doctoral dissertation, Monash University, Melbourne, Australia.

McCarthy, D.T. (2009). A traditional first flush assessment of *E. coli* in urban stormwater runoff. *Water Science and Technology*, 60(11): 2749-2757.

National Oceanic and Atmospheric Administration (NOAA). (2009). Vapor pressure calculator. http://www.srh.noaa.gov/epz/?n=wxcalc_vaporpressure (Nov. 18, 2009).

Sansalone, J.J., and S.G. Buchberger. (1997). Partitioning and first flush of metals in urban roadway storm water. *Journal of Environmental Engineering*, 123(2): 134-143.

Sansalone, J.J., and C. M. Cristina. (2004). First flush concepts for suspended and dissolved solids in small impervious watersheds. *Journal of Environmental Engineering*, 130(11): 1301-1314.

Selvakumar, A., and M. Borst. (2006). Variation of microorganism concentrations in urban stormwater runoff with land use and seasons. *Journal of Water and Health*, 4(1): 109-124.

State Climate Office of North Carolina (SCONC). (2009). 1971 – 2000 Climate Normals. http://www.nc-climate.ncsu.edu/cronos/normals.php?station=317079 (Nov. 18, 2009).

Stenstrom, M.K., G.S. Silverman, and T.A. Bursztynsky. (1984). Oil and grease in urban stormwaters. *Journal of Environmental Engineering*, 110(1): 58-72.

United States Environmental Protection Agency (USEPA). (2008). Water quality assessment and total maximum daily loads information (ATTAINS). http://www.epa.gov/waters/ir/index.html (November 29, 2008).

Wake County and City of Raleigh (WCCOR). (2010). Imaps. http://imaps.co.wake.nc.us/imaps/ (February 16, 2010).

Wanielista, M.P., and Y.A. Yousef. (1993). *Stormwater Management*, Wiley, New York.

Yacub, G.P., D.A. Castric, K.L. Stadterman-Knauer, M.J. Tobin, M. Blazina, T.N. Heineman, G.Y. Yee, L. Frazier. (2002). Evaluation of Colilert and Enterolert defined substrate methodology for wastewater applications. *Water Environment Research*, 74(2), 131 – 135.

4 Indicator Bacteria Removal in Storm-Water Best Management Practices in Charlotte, North Carolina

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4.1 Abstract

Water quality degradation due to pathogen pollution is a major concern in the United States. Stormwater runoff is an important contributor to the transport of indicator bacteria from urbanized watersheds to nearby surface waters. With TMDLs being established to reduce the export of indicator bacteria to surface waters, stormwater Best Management Practices (BMPs) may be an important tool in treating indicator bacteria in runoff. However, the ability of these systems to remove indicator bacteria is not well established. A study in Charlotte, North Carolina, monitored nine stormwater BMPs (1 wet pond, 2 stormwater wetlands, 2 dry detention basins, 1 bioretention area, and 3 proprietary devices) for fecal coliform and *Escherichia coli* (*E. coli*). A wet pond, two wetlands, a bioretention area, and a proprietary device all removed fecal coliform with an efficiency higher than 50%; however, only the wetlands and bioretention area had significantly different influent and effluent concentrations ($p<0.05$). For *E. coli*, only one of the wetlands and the bioretention area provided a concentration reduction greater than 50%, both of which had a significant difference in influent and effluent concentrations ($p<0.05$). Only one of the nine BMPs had a geometric mean effluent concentration of fecal coliform lower than the USEPA target value, while four of the nine BMPs had geometric mean effluent concentrations lower than USEPA standard for *E. coli*. This study showed that some BMPs may be useful for treatment of indicator bacteria; however, other BMPs did not perform well . Because wet, nutrient-rich environments exist in many stormwater BMPs, there is a potential for indicator bacteria to persist in these systems.

4.2 Introduction

Pathogen pollution is a contributor to water quality degradation and is an obstacle to the goal of the Clean Water Act "to restore and maintain the chemical, physical, and biological integrity of the nation's waters." In the United States Environmental Protection Agency's (USEPA 2002a) National Water Quality Inventory in 2000, 13% of the river and stream miles that were surveyed were impaired by indicator bacteria. Further, of the stream and river miles designated as impaired, either unable or partially unable to meet their designated use, more were impacted by indicator bacteria than by any other pollutant or stressor (USEPA 2002a). In light of the negative impact that indicator bacteria and other pollutants have on surface waters in the United States, Total Maximum Daily Loads (TMDLs) have been established to reach water quality goals in impaired water bodies.

Indicator species are used to test for the presence of harmful pathogens in surface waters. While these species may not be harmful to humans themselves, their presence in surface waters can indicate contamination from the fecal matter of warm-blooded animals. Fecal matter can contain harmful intestinal viruses, bacteria, and protozoa (Myers 2003). Various indicator bacteria have been used to assess water quality degradation due to pathogens including: total coliform, fecal coliform, *Escherichia coli* (*E. coli)*, and enterococci. In 1986, the USEPA's Ambient Water Quality Criteria for Bacteria report (USEPA 1986) discussed the merits of these various indicator species and set criteria whereby *E. coli* and enterococci were recommended for use as indicators in freshwater environments and enterococci were suggested as an indicator in marine environments. These criteria stated that for fresh waters designated for use as full body contact recreational waters, the geometric mean over a 30-day period should not exceed 126 /100 ml for *E. coli* and should not exceed 33 /100 ml for enterococci. For similarly designated marine waters, the geometric mean over a 30-day period should not exceed 35 /100 ml for enterococci.

A literature review by Wade et al. (2003) concluded that the USEPA bacteria standards set forth for marine waters was supported by the available literature. For fresh waters, Wade et al. (2003) indicated that *E. coli* were a more consistent predictor of gastrointestinal illness than any other indicator. Despite this, fecal coliform remains a commonly used bacteria indicator for surface waters. In 1976, the USEPA recommended fecal coliform standards where the log mean over a 30-

day period should not exceed 200 /100ml (colony forming units per 100 ml) and no more than 10 percent of the samples should exceed 400 /100ml. As of 2003, 18 states had adopted the *E. coli* standard for fresh water, 6 states adopted the enterococci standard for fresh waters, and 9 states had adopted the enterococci standard for marine waters (USEPA 2003a).

Stormwater runoff is an important transport mechanism for indicator bacteria to receiving waters. Indicator bacteria come from both human and animal (domestic and wild) sources, and are transported via runoff to nearby water bodies. A study by the Municipality of Anchorage Watershed Management Services (MOAWMS 2003) found that these transport mechanisms vary based on land use, type of stormwater conveyance system, and the degree of stream modification. The study by MOAWMS indicated that fecal coliform loading was high in runoff that originates from landscapes associated with densely urbanized areas drained by curb and gutter. Schoonover and Lockacy (2006) showed a similar trend in a study of 18 mixed land use watersheds in West Georgia and indicated that watersheds consisting of greater than 24% imperviousness exhibit higher fecal coliform concentrations than watersheds with impervious percentages less than 5% during both base and storm flow.

Stormwater runoff from urbanized areas can increase indicator bacteria concentrations in nearby surface waters, suggesting an increased risk to public health. This is a concern in both freshwater and ocean environments. A substantial amount of research has examined the impact of bacterial pollution on ocean environments, likely due to the economic and public safety concerns associated with shellfish waters and recreational ocean beaches. In Santa Monica Bay, California, Haile et al. (1999) showed an increased risk of health effects to swimmers located closer to storm drain outlets, noting that higher levels of bacterial indicators were found near the storm drain.

Indicator bacteria can be removed from or inactivated in surface waters and stormwater through a number of treatment mechanisms, such as ultraviolet light (from sunlight), sorption, sedimentation, and filtration. Additionally, as living organisms, various environmental factors, such as temperature, moisture conditions, and salinity, can impact the ability of indicator bacteria to survive in a given natural environment (USEPA 2001; Schueler 2000; Arnone 2007; Davies-Colley et al. 1994). Urban

stormwater is commonly treated by stormwater Best Management Practices (BMPs), each of which provides some combination of treatment mechanisms and provides a certain set of environmental conditions. Stormwater BMPs include wet ponds, dry detention basins, wetlands, bioretention areas, and proprietary devices. Proprietary, or manufactured, devices use some combination of baffles, swirl flow patterns, settling chambers, filtration and other means to separate floatable and settleable solids from stormwater runoff.

Although BMPs have been studied in detail for many pollutants, little peer-reviewed literature is available which documents their ability to remove or inactivate indicator bacteria. The majority of the BMP data associated with indicator bacteria removal is available in a database format through the International Stormwater BMP Database (ISBD) (USEPA 2003b; USEPA 2006). Based primarily on data entered into the ISBD, the USEPA (2003b) concluded that BMP performance with respect to indicator bacteria is less understood than for other pollutants. The data that have been collected have been primarily for sand filters, wetlands, and wet detention ponds. Further, the paper highlights the variable performance that initial studies have shown with respect to BMP indicator bacteria removal. This is a concern as stormwater BMPs are commonly used to achieve TMDLs in impaired surface waters. The functionality of stormwater BMPs for indicator bacteria removal is important in determining if non-point source indicator bacteria can be treated using these devices.

Although a number of studies have been performed on indicator bacteria removal in wetlands receiving wastewater (Karim et al. 2004; Vymazal 2004; Perkins and Hunter 2000; Ghermandi et al. 2007; Quiñónez-Diaz et al. 2001), few peer reviewed studies have been performed on indicator bacteria removal in wetlands receiving stormwater runoff. Birch et al. (2004) collected a limited number (4) of samples during high flow events at a stormwater wetland in Sydney, Australia. The mean fecal coliform removal in the wetland was 76% with a range of 26 – 98%. The average fecal coliform outflow concentration for each of the wetlands was higher than the USEPA target value of 200 /100ml. Similar mean removal was found by Davies and Bavor (2000) in a study of a stormwater wetland receiving residential stormwater in New South Wales, Australia. The wetland mean removal of fecal coliform (called thermotolerant coliform in the study), enterococci, and heterotrophic bacteria was 79%, 85%, and 87%, respectively. The mean effluent concentration of fecal coliform

was 3600 col/100ml. It should be noted that Sydney, Australia, and Charlotte, North Carolina, exhibit similar rainfall totals and distributions from month to month (ABM 2008; SCO - NC 2008).

A wet pond receiving residential stormwater runoff was also monitored in the study by Davies and Bavor (2000). Mean removal of fecal coliform (thermotolerant coliform), enterococci, and heterotrophic bacteria was -2.5%, 23%, and 22%, respectively. Davies and Bavor (2000) associated the poor performance of the wet pond, relative to the wetland, to its poor removal of fine clay particles, to which the bacteria were "predominately absorbed." The mean effluent concentration of fecal coliform was 8100 col/100ml. Research was also performed on three wet ponds in Wilmington, North Carolina, by Mallin et al. (2002). The ponds were sampled monthly, regardless of whether the pond discharge was base flow or storm flow. The authors did not report the percentage of samples associated with wet weather. The average fecal coliform removal in the three ponds was 56%, 86%, and -13%, and a correlation was observed between fecal coliform concentrations and rainfall occurring within 24 hours of a given pond being sampled. The average effluent fecal coliform concentrations for the three wet ponds was 70, 43, and 85 col/100ml, respectively; however, only one of the wet ponds had an average influent fecal coliform concentration higher than the USEPA targeted value of 200 /100ml (488 col/100ml). These studies suggest some variability in wet pond performance with regard to indicator bacteria removal.

A more detailed study was performed by Struck et al. (2008) in an evaluation of indicator bacteria removal in wetlands and wet ponds using mesocosms. Results of the study suggested that indicator organism concentrations decreased exponentially over time in mesocosms subjected to stormwater which had been manipulated to increase bacterial concentrations. Struck et al. (2008) also examined factors contributing to this decay, indicating that temperature, light exposure, time, and other effects can impact indicator bacteria concentrations in simulated stormwater wetlands and wet ponds. Other effects included oxygen-reduction potential, pH, dissolved oxygen, and conductivity.

A study by CALTRANS (2004) evaluated the performance of five dry extended detention basins. Four of the dry detention basins were unlined and one was lined with concrete. The average fecal coliform concentration reduction was -122% for the unlined basins and -12% for the lined basin.

Average effluent fecal coliform concentrations were 2000 MPN/100ml and 7500 MPN/100ml for the unlined and lined basins, respectively. Little discussion is provided as to the reason for the poor performance of these systems. Conversely, a study by Harper et al. (1999) evaluated the performance of a dry detention basin in Debary, Florida. The basin was drained via a perforated PVC pipe which was surrounded by sand. The overall system showed a 98% removal of fecal coliform.

Bioretention areas have not been studied in detail with respect to indicator bacteria. The bioretention area studied as part of the City of Charlotte Pilot BMP Program was documented extensively by Hunt et al. (2008), and no other field studies were found in which bioretention indicator bacteria removal was evaluated. However, a bioretention column study performed by Rusciano and Obropta (2007) examined indicator bacteria removal in simulated bioretention areas. The bioretention columns were loaded with diluted manure slurry with influent fecal coliform concentrations ranging from $2.3x10^7$ to $2.3x10^3$ CFU/100 ml. The average fecal coliform removal for 13 simulations over a 9 month period was approximately 96%, and leachate effluent mean concentrations ranged from $3.3x10^5$ to $2.0x10^1$ CFU/100 ml. Bioretention areas are expected to perform similarly to sand filters, which were evaluated for fecal coliform removal in a study by Barrett (2003). Five sand filters had a fecal coliform influent EMC of 11,200 MPN/100 ml and an effluent EMC of 3,900 MPN/100 ml, a reduction of 65%.

Zhang and Lulla (2006) studied two hydrodynamic separation devices in Providence, Rhode Island, for 12 storm events. Indicator bacteria removal in systems 1 and 2 were determined to be 42% and 62%, respectively, for *E. coli* and 73% and 39%, respectively, for fecal coliform. The study noted that sediments within the device had higher concentrations of indicator bacteria than the sump water, concluding that resuspension of indicator bacteria from captured sediments could occur, potentially reducing removal efficiency below that reported. Additionally, Zhang and Lulla (2006) concluded that low BOD concentrations (less than 10 mg/L), and thus low nutrient concentrations, in the sump water of the device would make indicator bacteria regeneration unlikely. A study by CALTRANS (2004) also evaluated the performance of a proprietary device, showing fecal coliform removal efficiencies of -121%. Little discussion is provided on the poor performance of the system.

Due to the limited amount of literature pertaining to indicator bacteria removal by stormwater BMPs, more research is needed to aid communities throughout the United States in reaching their target indicator bacteria TMDL. Determining which BMPs are capable of efficient indicator bacteria reduction will result in more effective watershed restoration programs. More specifically, if *E. coli* becomes established as the primary indicator bacteria in fresh water, *E. coli* sequestration and removal must be established for a suite of stormwater BMP types, including wet ponds, dry detention, stormwater wetlands, bioretention, and proprietary devices.

4.3 Materials and Methods

4.3.1 Description of Sites

The stormwater BMPs evaluated in this project were monitored as part of the Charlotte – Mecklenburg Stormwater Services (CMSS) Pilot BMP Program. This program was developed, in part, to evaluate various types of BMPs within the City of Charlotte, North Carolina, to gather local BMP performance data. As part of the Pilot BMP Program, grab samples were taken and analyzed for both fecal coliform and *E. coli* from 12 stormwater BMPs. A viable data set, chosen to be 6 or more storm events with paired influent and effluent samples, was collected for two dry detention basins, one pond, two stormwater wetlands, one bioretention area, and three proprietary BMPs. The characteristics of these BMPs are given in Table 4.1.

Table 4.1: Watershed and BMP Summaries

Site	Watershed Size (ha)	Description	Estimated Curve Number	Estimated BMP Surface Area (ha)
Dry Detention 1	2.4	Office Park - Buildings and Parking	85	0.04
Dry Detention 2	1.5	Office Park - Buildings and Parking	94	0.07
Pond	48.6	Residential	75	0.31
Wetland 1	21	Residential	80	0.25
Wetland 2	6.4	Residential and School	83	0.13
Bioretention	0.4	Municipal Parking Lot	98	0.02
Proprietary 1	0.3	Bus Maintenance Facility – Parking and Overhead Shelters	98	n/a
Proprietary 2	0.9	Bus Maintenance Facility – Parking and Overhead Shelters	98	n/a
Proprietary 3	0.9	Bus Maintenance Facility – Parking and Overhead Shelters	98	n/a

Stormwater BMPs are designed for a number of purposes. BMPs such as dry detention basins and ponds are normally designed to attenuate peak flows from larger storm events and are often constructed in relatively large watersheds. Bioretention areas and proprietary devices are often sited in small, highly impervious watersheds. Bioretention areas offer little mitigation of peak flows during extreme storm events, but provide temporary capture and treatment of smaller, "water quality" rain events. In North Carolina, and several other states, the water quality event is 2.5 cm (1 inch) of precipitation (NCDENR 2007). This is a common design parameter for determining the capture volume required for a given stormwater BMP. Proprietary devices are commonly placed in stormwater conveyance pipes and provide some treatment of runoff over a wide range of storm events using methods that allow the water to flow-through without being detained. Stormwater wetlands can be constructed to detain larger storm events in sizable watersheds; however, they are commonly designed to treat the water quality event, as was the case for the Charlotte stormwater wetlands. Due to variable intended function, design specifications for each type of BMP differ, making normalization problematic. However, these BMPs were selected because they were representative of the types of BMPs common to the City of Charlotte, NC, and elsewhere.

Dry detention basins fill with runoff during storm events and provide temporary detention while slowly draining in approximately 48 hours. The primary pollutant removal mechanism in these systems is sedimentation. Dry detention 1 (Figure 4.1a) received runoff from a 2.4 ha watershed comprised of an office park and its associated parking areas, landscape features and buildings. The dry detention facility was well vegetated with grass and had good sun exposure. There was some evidence of erosion and sedimentation within the facility. Dry detention 2 (Figure 4.1b) was sited in a similarly sized watershed, 1.5 ha, also comprised of an office park. Like Dry Detention 1, this facility had good sun exposure, was well vegetated with grass, and had evidence of some erosion and sedimentation. Both facilities appeared to be mowed frequently. CMSS staff noted the frequent presence of birds around the basins, with bird droppings found on the boxes which housed flow and water quality sampling equipment.

Wet ponds work on the principle of plug flow, whereby, influent runoff enters the pond and theoretically replaces captured runoff from prior events. Sedimentation in wet ponds is a major

pollutant removal mechanism, but some treatment is also provided via other mechanisms such as oxidation-reduction reactions, plant uptake, and adsorption due to contact among the soils, vegetation and captured stormwater. The Wet Pond that was monitored in this study (Figure 4.1c) was fed by a small, perennial stream. This pond received stormwater runoff from a 48.6 ha watershed that was primarily residential. This pond was likely not originally created for stormwater management and was constructed with no detention component. The estimated age of the pond was between 50 and 70 years old. Waterfowl were frequently observed at the pond during site visits. The pond was retrofitted in the late 1990's to include a littoral shelf; however, the shelf was not planted and exhibited little vegetation during the study period. Despite the presence of trees around the BMP, there was good sunlight exposure on the pond.

Wetlands are commonly installed as water quality devices, treating small (2.5 cm) storm events. These BMPs promote sedimentation like wet ponds, but provide more exposure of captured stormwater to wetland soils and plants in a shallow system. Wetland 1 (Figure 4.1d) received stormwater from an approximately 21 ha residential area. This wetland exhibited common wetland topography, and consisted predominantly of shallow water depths averaging 18 cm. During the course of the study, however, there was very little vegetation in the wetland, likely due to poor soil conditions, prolonged periods of high water levels due to slow drainage after storm events, and the impact of waterfowl grazing. This lack of vegetation resulted in a larger amount of full sun exposure to water in the wetland than would typically be expected for wetlands. Waterfowl, particularly ducks, were commonly observed at this site. Wetland 2 (Figure 4.1e) was constructed with similar topography, but exhibited exceptional plant growth. Wetland 2 received stormwater from a 6.4 ha watershed consisting of residential area and a school. This wetland had two inlets, thus, average influent fecal coliform and *E. coli* concentrations were calculated by weighting the grab samples at each inlet based on the proportion of the total flow they contributed. A description of the flow monitoring at Wetland 2 is in the "Monitoring Methods" section of this paper. A portion of the watershed was localized, draining to the wetland via overland flow. This overland flow was not monitored, which could represent some error for this site. Wildlife was observed at Wetland 2 during the study. Both wetlands were fed by watersheds containing a large amount of residential

area, likely meaning the presence of domestic animals, and leading to a bacterial contribution from pet waste (Young and Thackston 1999; Mallin et. al 2000).

(a) DD1 (b) DD2

(c) WP (d) WL1

(e) WL2 (f) BR

Figure 4.1: Illustration of (a) Dry Detention 1 (DD1), (b) Dry Detention 2 (DD2), (c) Wet Pond (WP), (d) Wetland 1 (WL1), (e) Wetland 2 (WL2), and (f) Bioretention (BR)

Bioretention areas function as filtration and infiltration BMPs. Stormwater enters the system and passes through a permeable soil media where pollutants are filtered, as is seen commonly in sand filter systems. The system may pond water as much as 6 to 12 inches; however, it is drained within 12 to 24 hours. The system is intended to dry out between storm events. The Bioretention monitored in this study (Figure 4.1f) received runoff from a highly impervious 0.4 ha parking lot. The parking lot was used primarily as parking for employees and visitors of the Mecklenburg County (NC) Social and Environment Services. This bioretention cell was studied and described in detail by Hunt et al. (2008). On at least one occasion, a diaper was observed in the parking lot, providing a potential source of bacteria to the BMP. Additionally, trees in the parking lot attracted birds, and evidence of bird droppings were observed by CMSS staff. Sun exposure in the BMP was fair, as it was limited by fairly dense vegetation.

All three proprietary systems were installed at the Charlotte Area Transit System Bus Operations Maintenance Facility. The watersheds were small (Table 4.1) but highly impervious, and consisted of bus parking areas and some overhead metal shelters. These systems were underground and thus received no sunlight. Proprietary 1 worked by passing runoff through a system where floatable and settleable solids were separated and captured. Proprietary 2 worked by forcing influent flows into a swirl pattern where settleable and floatable solids were forced to the center of the system and into a separation chamber. Proprietary 3 worked by routing stormwater through a series of chambers where floatable solids were captured and sedimentation occurred. In Proprietary 3, flows were controlled to allow treatment in each chamber during small storm events, while larger flows were treated only in one chamber before exiting the system.

4.3.2 Monitoring Methods

Due to the small sample hold times required of bacteriological samples, CMSS staff collected grab samples for fecal coliform and *E. coli* examinations (USEPA 2002b) from each site during rainfall events. The only site with more than one inlet was Wetland 2, where the influent bacteria concentration was estimated by weighting the two influent grab samples based on approximate proportions of flow. One inlet was monitored for flow by monitoring stage changes over a 120-degree v-notch weir using an ISCO Avalanche™ automated sampler equipped with an ISCO 730™

bubbler flow module. The second inlet was monitored for flow using an ISCO Avalanche™ automated sampler equipped with an ISCO 750™ area velocity flow module installed in a 24-inch reinforced concrete pipe.

Standard Method 9060 for microbiological examination (APHA 1998) was followed for sample collection. Samples were taken using disposable, sterilized sample bottles which contained tablets of sodium thiosulfate (a chlorine-neutralizing compound). A sample of at least 100 ml was taken and stored on ice while being transported to the Charlotte Mecklenburg Utilities Laboratory for analysis. A maximum hold time of 6 hours between sample collection and delivery to the laboratory was adhered to. Samples were tested for fecal coliform using Standard Method 9222D, while *E. coli* were examined using Standard Method 9223B (APHA, 1998). For fecal coliform, 12% of the influent samples and 21% of the effluent samples were either less than the Limit of Detection (LOD) or exceeded the maximum reporting limit (MRL). For *E. coli*, 38% of the influent samples and 33% of the effluent samples were either less than the LOD or exceeded the MRL.

One inherent source of error in analyzing samples for indicator bacteria is the dilution sometimes performed as part of the sample analysis procedure (USEPA, 2003c). Undiluted samples often contain indicator concentrations too high to be estimated using these analysis methods. To achieve test results which provide adequate readings of indicator bacteria, samples are often diluted to allow analysis of samples with higher concentrations. Unfortunately, stormwater samples have a wide range of indicator concentrations from storm to storm, as seen in this study. Therefore, a standard dilution is difficult to apply as the appropriate dilution differs among storms. For a given storm event, selecting the appropriate dilution is an iterative process, requiring that multiple analyses be performed. If an appropriate dilution is not selected, the analysis will provide some insight into actual concentration of a given pollutant, but the results will have a “maximum reporting limit” (MRL) or “Limit of Detection” (LOD) meaning that the analysis can only conclude that the actual concentration is above or below some reporting limit as constrained by the dilution that was selected. For fecal coliform, the LOD was typically 100 col/100 ml and the MRL varied based on the dilution used for a given sample. For *E. coli*, the LOD was 10 MPN/100 ml and the MRL

was 2400 MPN/100 ml. Data are analyzed herein using the values at the reporting limit without manipulation.

Bacteria grab samples were collected at the various sites between March 2004 and October 2006. A grab sample was taken from both the inlet and outlet of each BMP per precipitation event. An effort was made to take grab samples during the rising limb of the storm event, but this was not always the case. The monitoring period and number of samples collected at each site varied (Table 4.2). *E. coli* were not initially tested for in the bacteria grab samples, but were later added as a parameter.

Table 4.2: Monitoring Period and Number of Samples Taken at Each Study Location

Site	Start	End	Number of Sample Tested For Fecal Coliform	Number of Samples Tested For *E. coli*
Dry Detention 1	Feb-05	Jul-06	9	9
Dry Detention 2	Jan-05	Dec-05	12	12
Wet Pond	Aug-04	Apr-06	14	10
Wetland 1	Mar-04	Jun-05	9	6
Wetland 2	Sep-04	Dec-05	15	10
Bioretention	Aug-04	Mar-06	19	14
Proprietary 1	Oct-05	Oct-06	7	7
Proprietary 2	Oct-05	Oct-06	6	6
Proprietary 3	Oct-05	Oct-06	6	6

4.3.3 Statistical Analysis

To adequately describe the indicator bacteria sequestration and removal performance of each BMP, various analyses were performed. The mean concentration method was used to gain some understanding of each BMP's ability to remove the influent indicator bacteria. This method is similar to that used in determining the efficiency ratio; however, in determining the efficiency ratio, event mean concentrations are required (USEPA, 2002b). Obviously, when analyzing data generated from grab samples, this is not possible.

Although evaluating BMP efficiency based *solely* on methods such as the mean concentration method or the efficiency ratio is not suggested (Urbonas, 2000; Strecker et al., 2001; USEPA, 2002b), these methods do provide a simple estimation of the percent of a given pollutant treated by the

BMP, on a concentration basis, relative to what the practice received during the storms that were monitored. There are inherent errors in these methods, as the data set may include samples from storm events that are abnormal in some manner, and thus artificially raise or lower the true BMP efficiency. Additionally, grab samples may not accurately portray BMP effectiveness if pollutant concentrations in the influent or effluent vary throughout the course of the storm. The mean concentration method was used to generate a concentration reduction efficiency (CR); however, geometric means were used in lieu of arithmetic means as is common in indicator bacteria data manipulation (Equation 1).

$$\text{Equation 1: } CR = 1 - \left(\frac{Geometric\_Average\_Outlet\_Concentration}{Geometric\_Average\_Inlet\_Concentration}\right)$$

To supplement the mean concentration method, the effluent probability method was also employed (USEPA, 2002b). A Kolmogorov- Smirnov test was performed to determine a usable distribution of the data prior to additional statistical analysis. As part of the effluent probability method, data were transformed into the correct distribution and were tested for significant differences between the influent and effluent bacteria concentrations using a non-parametric Wilcoxon signed rank test. Probability plots were generated to evaluate BMP performance over the entire concentration spectrum that was observed in the data set (Burton and Pitt, 2002). Lastly, the geometric mean effluent concentrations of fecal coliform and *E. coli* leaving each site were compared to the maximum 30-day geometric mean for each indicator as established by the USEPA for full body contact (USEPA 1986; USEPA 1976).

The LOD and MRL present for the samples likely had some impact on the results of these analyses. In general, fecal coliform data were most often affected by the LOD, generally 100 col / 100 ml as discussed above. *E. coli* data were most often affected by an MRL of 2400 MPN / 100 ml. The impact of these limits were more prevalent in some of the BMPs that others. The MRL associated with *E. coli* is considered more problematic, as there is no way to tell how high the actual indicator bacteria count is above 2400 MPN / 100 ml. For fecal coliform, the minimum reading must be somewhere between 0 and 100 col / 100 ml. The use of geometric means and non-parametric statistics lessens

the impact of the LOD and MRL, as they tend to be less sensitive to extreme observations. This is important as the LOD and MRL place lower and upper limitations on such observations.

4.4 Results and Discussion

4.4.1 Concentration Reduction Efficiency

CR values were developed for each site for both fecal coliform and *E. coli* (Table 4.3). The CR shows which BMPs were potentially adding bacteria to the stormwater drainage system. Any indicator bacteria increase was potentially due to either animal activity or from bacteria entering the flow stream from within the BMPs.

Table 4.3: Indicator Bacteria Concentration Reduction (CR) Efficiency for BMPs in Charlotte, NC.

BMP Type	Number of Fecal Samples	Efficiency Fecal Coliform (%)	Number of *E. coli* Samples	Efficiency *E. coli* (%)
Dry Detention 1	9	-0.45[1]	9	-0.22
Dry Detention 2	12	-0.20	12	0.00
Wet Pond	14	0.70	10	0.46
Wetland 1	9	0.98	6	0.96
Wetland 2	15	0.56	10	0.33
Bioretention	19	0.89	14	0.92
Proprietary 1	7	0.59	7	-0.02
Proprietary 2	6	-0.57	6	-2.69
Proprietary 3	6	-0.62	6	-0.07

1: Negative values indicate an increase in concentration

For the majority of BMPs, a similar reduction (or addition) in concentration was noted for both fecal coliform and *E. coli*; however, some BMPs exhibited dramatically different concentration reductions for these two indicators. This was possibly due, in part, to the difference in the number of samples taken for each indicator bacteria at a given site; however, even for sites with the same number of fecal coliform and *E. coli* samples, variations in the CR calculated for each indicator bacteria existed (such as Proprietary 1). This indicates that data generated for BMP removal of fecal coliform may not be consistent with BMP removal of *E. coli* in all cases. A study by Struck et al. (2008) on wetland and wet pond mesocosms showed various indicator bacteria may have different inactivation rates as

a result of various environmental factors. These factors included temperature, sunlight, and sedimentation.

For fecal coliform, the Wet Pond, Wetland 1, Wetland 2, Bioretention, and Proprietary 1 exhibited greater than 50% removal. The fecal coliform removal determined for Wetland 1 and Wetland 2, 0.98 and 0.56, was similar to that found by Birch et al. (2004). Conversely, only one of the three wet ponds studied by Mallin et al. (2002) showed fecal coliform removal equal to or greater than 70%. It should be noted that the fecal coliform CR determined for the Bioretention (0.89) was higher than the reduction (65%) reported for sand filters by Barrett (2003). For *E. coli*, only Wetland 1 and the Bioretention provided high (> 50%) concentration reductions, with the wet pond providing slightly lower reductions with a CR of 0.46.

Overall, Wetland 1 and the Bioretention seemed to be the most proficient at reducing influent concentrations of both kinds of bacteria. Wetland 1 had a substantial amount of sun exposure due to poor vegetation establishment, possibly leading to higher die off rates. Additionally, stormwater wetlands and bioretention areas facilitate sedimentation and, in the case of bioretention, filtration and relatively low soil moisture. This assertion is supported by Kibbey et al. (1978) who showed that soils even at field capacity had higher rates of bacteria die off than those that stayed saturated.

The poorest performing BMPs were the two dry detention basins. Both basins showed negative removal of fecal coliform, similar results to those of CALTRANS (2004). Although the basins had good sun exposure, they remained moist for a substantial period of time after each rain event per observation by CMSS staff. It is possible that the wet soil provided an environment where the indicator bacteria could survive for an extended period of time, as was the case in a study performed by Karim et al. (2004) on wetlands receiving wastewater. Karim et al. (2004) found that sediments within the wetland provided an environment where bacterial survival was prolonged. Animal activity was also noted in the basins by CMSS staff, most notably, bird feces was observed. It was likely that the dry detention basins were attracting animals and potentially providing an environment where indicator bacteria could persist.

Due to the limited number of samples collected at the site, evaluation of the proprietary devices was minimal. Initial results showed an increase in fecal coliform at two of the three devices and increases in *E. coli* at each site. Similar fecal coliform increases were determined for a proprietary system monitored by CALTRANS (2004). There was a consistent source of water and nutrients in the two proprietary devices which may have allowed the bacteria to persist and possibly be exported from the system during future rain events. It should be noted that Proprietary 1 performed well with respect to fecal coliform concentration reduction and likely would have had significantly different influent and effluent concentrations if more storm events had been collected at the site. However, Proprietary 1 performed poorly from a removal efficiency standpoint with respect to *E. coli*. It should be noted that the proprietary devices, particularly Proprietary 1 and 2, had low influent concentrations of both fecal coliform and *E. coli* relative to the other BMPs, potentially contributing to the low CRs for these systems. It should also be noted that these proprietary systems were not filter-based, as is the case with some proprietary devices.

4.4.2 Wilcoxon Signed Rank Test

The results of the Wilcoxon signed rank test supported the concentration reduction efficiency observations with the exception of fecal coliform removal in Proprietary 1. For fecal coliform, the influent concentration was significantly higher ($P<0.05$) than the effluent concentration for the two wetlands and the bioretention area (Table 4.4). For *E. coli*, only Wetland 1 and the Bioretention had significantly ($P<0.05$) higher influent than effluent concentrations (Table 4.5).

Table 4.4: Wilcoxon Signed Rank Results for Fecal Coliform

BMP Type	Distribution	Rank Sign	Significant?
Dry Detention 1	Lognormal	0.1484	No
Dry Detention 2	Lognormal	0.4131	No
Wet Pond	Lognormal	0.1099	No
Wetland 1	Lognormal	0.0039	**Yes**
Wetland 2	Lognormal	0.0132	**Yes**
Bioretention	Lognormal	0.0001	**Yes**
Proprietary 1	Lognormal	0.1250	No
Proprietary 2	Lognormal	0.2500	No
Proprietary 3	Lognormal	0.3750	No

Table 4.5: Wilcoxon Signed Rank Results for *E. coli*

BMP Type	Distribution	Rank Sign	Significant?
Dry Detention 1	Normal	0.1484	No
Dry Detention 2	Lognormal	0.4131	No
Wet Pond	Lognormal	0.1099	No
Wetland 1	Lognormal	0.0039	**Yes**
Wetland 2	Lognormal	0.4609	No
Bioretention	Lognormal	0.0015	**Yes**
Proprietary 1	Lognormal	0.1250	No
Proprietary 2	Lognormal	0.2500	No
Proprietary 3	Lognormal	0.3750	No

4.4.3 Influent and Effluent Probability Plots

Probability plots (Figure 4.2a-4.2i, 4.3a-4.3i) were used to compare BMP performance (effluent concentration) for a wide range of influent concentrations. Figure 4.2 shows the fecal coliform probably plots for each BMP. The plots for the Bioretention and Wetland 1 demonstrate the effectiveness of these BMPs over a wide range of influent concentrations. Samples at the outlet of the Bioretention frequently were below the LOD. The effluent fecal concentrations of Wetland 1 were also consistently low. At very high influent concentrations, the Bioretention seemed to show reduced performance; however, a similar trend was not observed in Wetland 1, which performed well at all influent concentrations.

The probability plots for the dry detention basins (Figures 4.2a and 4.2b) showed inconsistent performance throughout most concentrations, with increases noted at low concentrations. Two of the proprietary devices also typically performed poorly (Figures 4.2h and 4.2i), showing little to no difference between influent and effluent concentrations. At high concentrations, Proprietary 1 seemed to function effectively; however, proprietary devices 2 and 3 showed decreased performance at high concentrations. Probability plots for Proprietary 1 and 2 (Figures 4.2g and 4.2h, respectively) showed that the fecal coliform influent concentration for each was much lower relative to the other BMPs tested, probably limiting the device's ability to improve concentrations. The Wet Pond (Figure 4.2c) and Wetland 2 (Figure 4.2e) exhibited modest fecal coliform concentration improvements throughout the entire plot, but the Wet Pond provided more consistent removal among the various influent concentrations, particularly at low concentrations. The Wet Pond and

Wetland 2 generally produced effluent fecal coliform concentrations well above the 200 /100 ml standard.

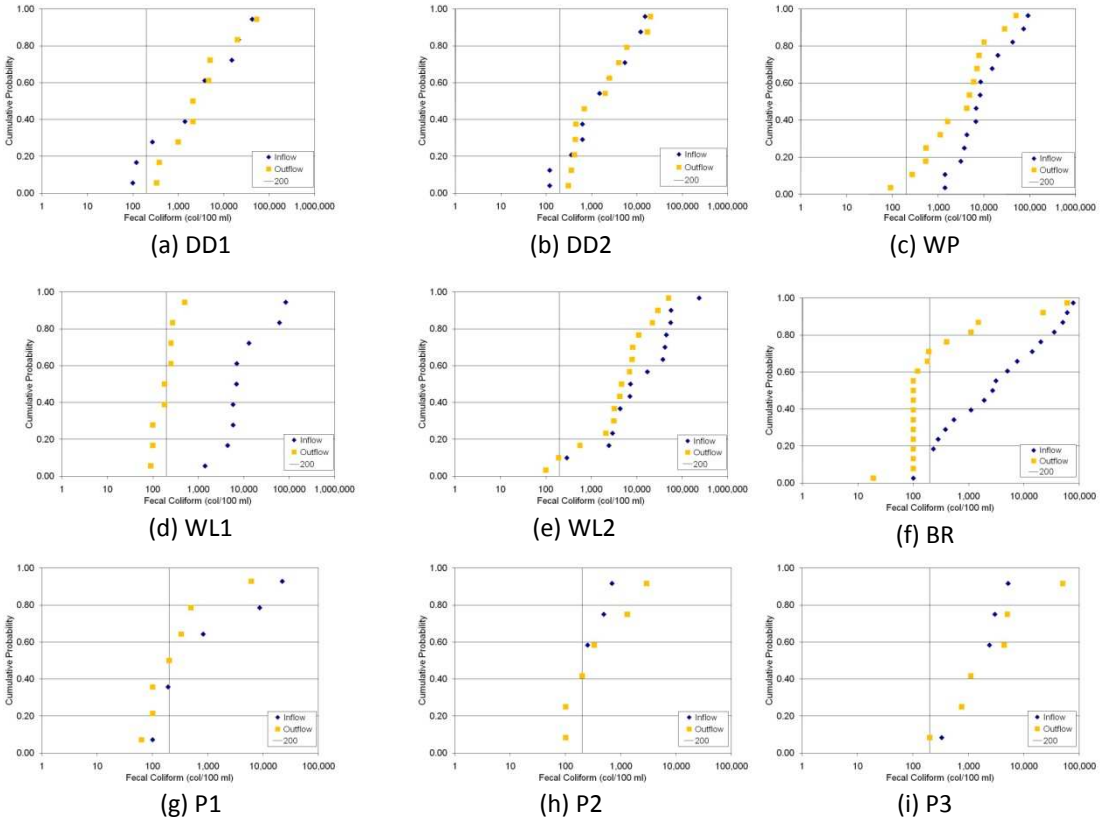

Figure 4.2: Fecal Coliform Influent and Effluent Probability Plots for (a) Dry Detention 1(DD1), (b) Dry Detention 2 (DD2), (c) Wet Pond (WP), (d) Wetland 1(WL1), (e) Wetland 2 (WL2), (f) Bioretention (BR), (g) Proprietary 1 (P1), (h) Proprietary 2 (P2), (i) Proprietary 3 (P3)

Probability plots were also generated for each BMP for *E. coli* (Figure 4.3). These plots illustrate the difficulty of bacteria analysis when reporting limits exist. As seen on plots 3a through 3e, an MRL was normally present at an *E. coli* count of 2400 MPN/100ml. Reporting limits can make analysis difficult, particularly when influent and effluent concentrations are similar. On such plots, evaluating the performance of a system when receiving high *E. coli* concentrations can be difficult or impossible, as no distinction can be made between the influent and effluent concentrations.

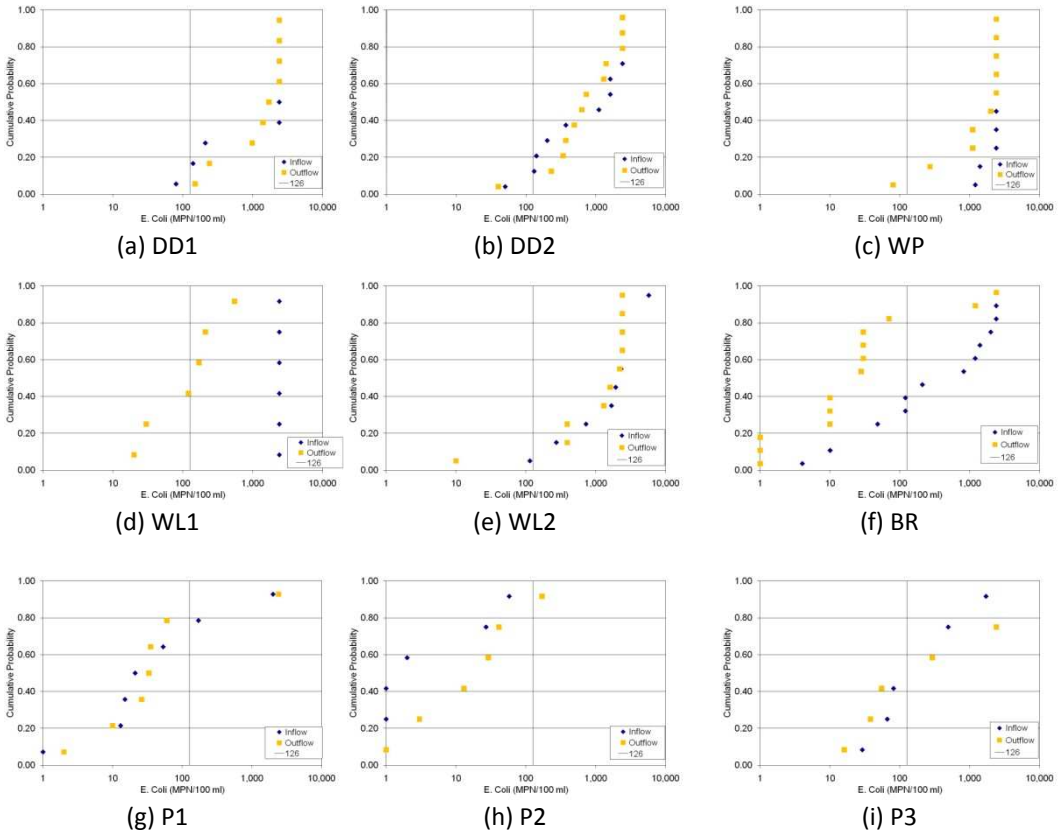

Figure 4.3: *E. coli* Influent and Effluent Probability Plots for (a) Dry Detention 1 (DD1), (b) Dry Detention 2 (DD2), (c) Wet Pond (WP), (d) Wetland 1 (WL1), (e) Wetland 2 (WL2), (f) Bioretention (BR), (g) Proprietary 1 (P1), (h) Proprietary 2 (P2), (i) Proprietary 3 (P3)

Despite the reporting limits evident in these probability plots, the Bioretention and Wetland 1 seemed to perform well with respect to *E. coli*, as was the case with fecal coliform. Both systems showed consistent performance over a range of influent *E. coli* concentrations. Wetland 1 received influent *E. coli* concentrations that were consistently at or above the reporting limit, whereas the Bioretention received a more diverse set of influent concentrations. At higher concentrations, the Bioretention seemed to show some reduction in efficiency, also seen in the probability plots generated for fecal coliform. The Wet Pond performed well when receiving low influent *E. coli* concentrations; however, as influent concentrations increased to reporting limits, effluent flows increased towards reporting limits as well. The reporting limit made evaluation of this practice at high concentrations impossible. Generally, the dry detention basins, Wetland 2, and the 3

proprietary devices did not perform well with respect to *E. coli* sequestration and removal. Effluent *E. coli* concentrations were similar to or higher than influent concentrations for these BMPs. However, the findings for Proprietary 1, 2 and 3 must be tempered by low influent *E. coli* concentrations noted in the probability plots for each. Particularly for Proprietary 1 and 2, influent *E. coli* concentrations were often below the regulatory standard of 126 / 100 ml.

4.4.4 Geometric Mean Effluent Concentration Analysis

Currently, water quality standards related to bacteria are concentration based. The USEPA recommended standard for fecal coliform is 200 /100 ml. Likewise, the recommended standard *E. coli* concentration is 126/100 ml. BMP geometric mean effluent concentrations were compared to these target values (Table 4.6). For fecal coliform, only one BMP had a geometric mean effluent concentration below or equal to the target value, Wetland 1. Five of the nine effluent samples from Wetland 1 were at or below 200 col/100 ml. The Bioretention performed fairly well in terms of this analysis (geometric mean effluent of 258 col/100 ml), with 14 of 19 samples being less than or equal to 200 col/100 ml. Although the geometric mean effluent of the Bioretention did not meet targeted values, the median effluent fecal coliform concentration of the Bioretention was calculated to be 100 col/100 ml, indicating some promise with respect to this system. Lastly, Proprietary 1 performed fairly well with a geometric mean effluent concentration of 277 col/100 ml. The median effluent concentration for Proprietary 1 was 200 ml/100 col, indicating relatively good performance. A small sample set for Proprietary 1 limits the ability to make generalizations about its ability to achieve targeted fecal coliform concentrations.

Four BMPs had geometric mean effluent *E. coli* concentrations below the USEPA target value of 126 /100 ml, Wetland 1, Bioretention, Proprietary 1, and Proprietary 2. Of these BMPs, only Wetland 1 and the Bioretention had a geometric mean effluent concentration lower than its geometric mean influent concentration. Of the 14 samples taken from the Bioretention, 12 had geometric mean effluent concentrations less than the target value. Of the 14 influent samples taken at the Bioretention, 6 were below the USEPA target value of 126 /100 ml. For *E. coli*, the Bioretention received relatively low influent concentrations. Wetland 1 also exhibited the ability to reduce influent *E. coli* concentrations, with 3 of 6 samples being below the targeted value. Of the BMPs

with geometric mean effluent concentrations lower than the standard, Wetland 1 had the highest geometric mean influent concentration. None of the influent samples taken from Proprietary 2 were higher than the target value, and only 2 of the 7 samples taken at Proprietary 1 were higher than the target value, again highlighting the relatively clean quality of inflow to the proprietary devices, which potentially impacted the results for these systems. The small number of *E. coli* samples taken at proprietary devices 1, 2, 3 and Wetland 1 limited the ability to make generalizations on their performance.

Table 4.6: Geometric Mean Influent and Effluent Fecal Coliform and *E. coli* Concentrations

BMP Type	Fecal Coliform Concentrations (col/100 ml)			*E. coli* Concentrations (MPN/100 ml)		
	Geometric Mean Influent	Geometric Mean Effluent	% of effluent samples under 200 col/100 ml	Geometric Mean Influent	Geometric Mean Effluent	% of effluent samples under 126 MPN/100 ml
Dry Detention 1	1985	2873	0	915	1121	0
Dry Detention 2	1327	1590	0	655	658	8
Wet Pond	9033	2703	7	2122	1153	10
Wetland 1	9560	184	56	2400	106	50
Wetland 2	8724	3874	13	1295	864	10
Bioretention	2420	258	74	241	20	86
Proprietary 1	667	277	43	36	37	71
Proprietary 2	235	368	50	4	14	83
Proprietary 3	1472	2379	0	183	196	50

4.5 Conclusions

The results of this study support others in literature that urban watersheds are a non-point source of bacterial pollution in surface waters (Schoonover and Lockacy 2006, Mallin et al. 2000, Tufford and Marshall 2002). Even in watersheds consisting primarily of parking lots, concentrations of fecal coliform and *E. coli* entering BMPs can be higher than government (USEPA 1976, USEPA 1986) recommended maximum values, indicating the need for treatment. Although conclusions are limited somewhat by the LOD and MRL present in the data, the findings from this study suggest that some stormwater BMPs may effectively sequester and remove indicator bacteria. In particular, bioretention areas and some types of wetlands showed promise in bacterial treatment. A small-sized sample set, and low influent concentrations limited evaluation of the proprietary devices.

Bioretention significantly ($P<0.05$) reduced both fecal coliform and *E. coli* concentrations from the inlet to the outlet with concentration reduction efficiencies of 0.89 and 0.92, respectively. Wetland 1, which performed better than Wetland 2, was atypical due to its lack of vegetated growth. The shallow water depths present in Wetland 1 (15 – 45 cm) and minimal vegetative coverage likely led to more sun exposure, and potentially ultraviolet light penetration, to the wetland bottom than would normally be expected in a stormwater wetland. This high sun exposure possibly led to increased inactivation of indicator bacteria in the wetland in between storm events and during the slow drawdown after an event.

If the proper environment exists, it is also possible that stormwater BMPs can be sources of indicator bacteria. This is likely due to both animal activity and to indicator bacteria persistence within BMPs. This was potentially the case for the two dry detention basins as well as two of the proprietary BMP systems. This was also likely the case in the wetlands and wet pond; however, removal mechanisms within these BMPs resulted in a net reduction of indicator organisms. It should be noted that relatively low concentrations of fecal coliform and *E. coli* entered two of the three proprietary systems, which likely reduced the ability of these BMPs to further lower indicator bacteria concentrations.

Although positive concentration reductions were achieved by BMPs for both fecal coliform (5 of 9 BMPs) and *E. coli* (6 of 9 BMPs), only Wetland 1 provided a positive concentration reduction and a geometric mean effluent concentration lower than USEPA targeted concentrations for both fecal coliform and *E. coli*. Good light exposure at this site likely contributed to this performance. Of the nine BMPs studied, only Bioretention employed a filtering removal mechanism. It also was the driest BMP. These two factors, along with sun exposure, seemed to indicate it as a potential option for treating indicator bacteria. Although geometric mean effluent concentrations of fecal coliform were higher than the standard, median effluent concentrations were lower, and both median and geometric mean effluent *E. coli* concentrations were lower than the standard for this practice. Some bias might be present because the Bioretention received runoff with somewhat lower influent concentrations than either of the wetlands or the wet pond. The proprietary systems produced relatively low geometric mean effluent concentrations, but a small sample set and low influent

concentrations of *E. coli* limited their analysis. Probability plots generated for both Wetland 1 and for Bioretention indicate that there may be reduced effectiveness of these practices at high inlet indicator bacteria concentrations.

While some conclusions can be drawn from this study, only one or two examples of each BMP type where monitored with grab samples, limiting the ability to make generalizations. Additional study should be performed to evaluate the effectiveness of various BMP types in detail. Further study is also necessary to determine if bioretention effluent can reach USEPA targeted values when receiving high concentrations of indicator bacteria, and to evaluate proprietary systems with higher influent concentrations of fecal coliform and *E. coli* over a larger number of events. Analysis of proprietary systems which employ filtration would also be beneficial.

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4.7 References

American Public Health Association. (1998). "Standard Methods for the Examination of Water and Waste Water", 20th edition, New York, NY.

Arnone, R.D. and J.P. Walling. (2007). "Waterborne pathogens in urban watersheds." *Journal of Water and Health*, 5(1), 149-162.

Australian Bureau of Meteorology (ABM). (2008). "Climate Statistics for Australian Locations." <http://www.bom.gov.au/climate/averages/tables/cw_066037.shtml> (Jan. 21, 2008).

Barrett, M.E. (2003). "Performance, cost, and maintenance requirements of Austin sand filters." *Journal of Water Resources Planning and Management*, 129(3), 234-242.

Birch, G.F., C. Matthai, M.S. Fazeli, and J.Y. Suh. (2004). “Efficiency of a constructed wetland in removing contaminants from stormwater.” *Wetlands*, 24(2), 459-466.

Burton, G.A. and R.E. Pitt. (2002). “Stormwater Effects Handbook: A Toolbox for Watershed Managers, Scientists, and Engineers.” CRC Press, LLC, Boca Raton, Fl.

California Department of Transportation (CALTRANS). (2004). “BMP pilot retrofit program – final report.” CTSW-RT-01-050. Division of Environmental Analysis, Sacramento, Ca.

Davies, C.M. and H.J. Bavor. (2000). “The fate of stormwater-associated bacteria in constructed wetland and water pollution control pond systems.” *Journal of Applied Microbiology*, 89, 349-360.

Davies-Colley, R.J., R.G. Bell, and A.M. Donnison. (1994). “Sunlight Inactivation of Enterococci and Fecal Coliforms in Sewage Effluent Diluted in Seawater.” *Applied and Environmental Microbiology*, 60(6), 2049-2058.

Ghermandi, A., D. Bixio, P. Traverso, I. Cersosimo, and C. Thoeye. (2007). “The removal of pathogens in surface-flow constructed wetlands and its implications for water reuse.” *Water Science and Technology*, 56(3), 207-216.

Haile, R.W., J. Witte, M. Gold, R. Cressey, C. McGee, R.C. Millikan, A. Glasser, N. Harawa, C. Ervin, P. Harmon, J. Harper, J. Dermand, J. Alamillo, K. Barrett, M. Nides, and G. Wang. (1999). “The health effects of swimming in ocean water contaminated by storm drain runoff.” *Epidemiology*, 10(4), 355-363.

Harper, H.H., J.L. Herr, D. Baker, and E. H. Livingston. (1999). “Performance evaluation of dry detention stormwater management systems.” Proc. Sixth Biennial Stormwater Research and Watershed Management Conference, Southwest Florida Water Management District, Tampa, Fl., 162 – 178.

Hunt, W.F., J.T. Smith, S.J. Jadlocki, J.M. Hathaway, and P.R. Eubanks. (2008). “Pollutant removal and peak flow mitigation by a bioretention cell in urban Charlotte, NC.” *Journal of Environmental Engineering*, 134(5), 403 – 408.

Karim, M.R., F.D. Manshadi, M.M. Karpiscak, and C.P. Gerba. (2004). “The persistence and removal of enteric pathogens in constructed wetlands.” *Water Research*, 38, 1831-1837.

Kibbey, H.J., C. Hagedorn, and E.L. McCoy. (1978). “Use of fecal streptococci as indicators of pollution in soil.” *Applied and Environmental Microbiology*, 35(4), 711-717.

Mallin, M.A., K.E. Williams, E.C. Esham, and R.P. Lowe. (2000). “Effect of human development on bacteriological water quality in coastal watersheds.” *Ecological Applications*, 10(4), 1047-1056.

Mallin, M.A., S.H. Ensign, T.L. Wheeler, and D.B. Mayes. (2002). “Pollutant removal efficacy of three wet detention ponds.” *Journal of Environmental Quality*, 31, 654-660.

Municipality of Anchorage Watershed Management Services (MOAWMS). (2003). “Fecal Coliform in Anchorage Streams: Sources and Transport Processes.” Document No. APg03001, Anchorage, AK.

Myers, D.N., D.M. Stoeckel, R.N. Bushon, D.S. Francy, and A.M.G. Brady. (2007). “Fecal indicator bacteria.” U.S. Geological Survey Techniques of Water-Resources Investigations, book 9, chap. A7, section 7.1

North Carolina Department of Environment and Natural Resources (NCDENR). (2007). “Stormwater Best Management Practices Manual.” Raleigh, NC.

Perkins, J. and C. Hunter. (2000). “Removal of enteric bacteria in a surface flow constructed wetland in Yorkshire, England.” *Water Research*, 34(6), 1941-1947.

Quiñónez-Diaz, M., M.M. Karpiscak, E.D. Ellman, and C.P. Gerba. (2001). “Removal of pathogenic and indicator microorganisms by a constructed wetland receiving untreated domestic wastewater.”*Journal of Environmental Science and Health*, 36(7), 1311-1320.

Rusciano, G.M. and C.C. Obropta. (2007). “Bioretention column ctudy: fecal coliform and total suspended solids reductions.” *Transactions of the ASABE*, 50(4), 1261-1269.

Schoonover, J.E. and B.G. Lockacy. (2006). “Land cover impacts on stream nutrients and fecal coliform in the lower piedmont of West Georgia.” *Journal of Hydrology*, 331,371-382.

Schueler, T. (2000). “Microbes and Urban Watersheds: Ways to Kill ‘Em.” Technical Note 67. *Watershed Protection Techniques*. 3(1):566-574.

State Climate Office of North Carolina (SCO-NC). 2008. “1971 – 2000 Climate Normals – Charlotte Douglas Ap.” < http://www.nc-climate.ncsu.edu/cronos/normals.php?station=311690l> (Jan. 21, 2008).

Strecker, E.W., M.M. Quigler, B.R. Urbonas, J.E. Jones, and J.K. Clary. (2001). “Determining Urban Stormwater BMP Effectiveness.” *Journal of Water Resources Planning and Management*, 127,144-149.

Struck, S.D., A. Selvakumar, and M. Borst. (2008). “Prediction of effluent quality from retention ponds and constructed wetlands for managing bacterial stressors in storm-water runoff.” *Journal of Irrigation and Drainage Engineering*, 134(5), 567 – 578.

Tufford, D.L. and W.D. Marshall. (2002). “Fecal coliform source assessment in a small, mixed land use watershed.” *Journal of the American Water Resources Association*, 138(6), 1625-1635.

Urbonas, B.R. (2000). “Assessment of stormwater best management practice effectiveness.” (chapter 7). *In:* (eds). Heaney, J.P., R. Pitt, R. Field. “Innovative Urban Wet-Weather Flow Management Systems.” EPA/600/R-99/029, Washington, DC.

United States Environmental Protection Agency (USEPA). (1976). “Quality criteria for water.” EPA-440/9-76-023, Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA). (1986). “Ambient water quality criteria for bacteria – 1986.” EPA 440/5-84-002. Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA). (2001). “Protocol for developing pathogen TMDLs.” EPA-841-R-00-002. Office of Water, Washington, DC.

United States Environmental Protection (USEPA). (2002a). “National Water Quality Inventory 2000 Report.” EPA-841-R-02-001. Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA) and American Society of Civil Engineers. (2002b). “Urban Stormwater BMP Performance Monitoring: A Guidance Manual for Meeting the National Stormwater Database Requirements.” EPA-821-B-02-001, Washington, DC.

United States Environmental Protection Agency (USEPA). (2003a). “Bacterial water quality standards for recreational waters: status report.” EPA-823-R-03-008. Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA). (2003b). “Managing urban watershed pathogen contamination.” EPA/600/R-03/111. Office of Research and Development, Cincinnati, OH.

United States Environmental Protection Agency (USEPA). (2003c). “Control and Pathogens and Vector Attraction in Sewage Sludge.” EPA/625/R-92/013, Office of Research and Development, Cincinnati, OH

Vymazal, J. (2004). “Removal of enteric bacteria in constructed treatment wetlands with emergent macrophytes: a review.” *Journal of Environmental Science and Health*, 40, 1355-1367.

Wade, T.J., N. Pai, J. Eisenberg, and J.M. Colford. (2003). “Do U.S. Environmental Protection Agency water quality guidelines for recreational waters prevent gastrointestinal illness? A systematic review and meta-analysis.” *Environmental Health Perspectives*, 111(8), 1102-1109.

Young, K.D., and E.L. Thackston. (1999). “Housing density and bacterial loading in urban streams.” *Journal of Environmental Engineering*, 125, 1177-1180.

Winer, R. (2000). “National Pollutant Removal Performance Database for Stormwater Treatment Practices.” 2nd Edition, Center for Watershed Protection, Ellicott City, MD.

Zhang, X. and M. Lulla. (2006). “Evaluation of pathogenic indicator bacteria in structural best management practices.” *Journal of Environmental Science and Health Part A*, 41, 2483-2493.

5 Indicator Bacteria Performance of Stormwater Control Measures in Wilmington, NC

5.1 Abstract

Indicator bacteria are a common source of impairment in surface waters in the United States. Urban stormwater runoff has been identified as a contributor to elevated indicator bacteria concentrations. Six Stormwater Control Measures (SCMs) were monitored in Wilmington, NC, for *Escherichia coli* (*E. coli*) and enterococci. Monitored SCMs included two stormwater wet ponds, two bioretention cells, and two stormwater wetlands. Sandier watersheds in Wilmington potentially lead to differences in SCM performance for indicator bacteria compared to SCMs implemented in clayey watersheds. Results showed *E. coli* and enterococci concentration reductions between 70 and 98% for the two wet ponds and a bioretention cell with a 60 cm deep fill media. Other SCMs showed poor removal of indicator bacteria, in some cases negative, with stormwater wetlands performing the poorest overall for the three SCM types. Further analysis showed that SCMs with high concentration reductions tended to have geometric mean effluent concentrations lower than the United States Environmental Protection Agency's (USEPA) target surface water concentration for *E. coli*. Conversely, no SCM had a geometric mean effluent enterococci concentration lower than the USEPA target value. SCM geometric mean effluent concentrations were typically higher during North Carolina's swimming season between the beginning of April and the end of October, although no statistically significant relationship could be found ($p < 0.05$). Despite a lack of statistically significant relationships, the potential for higher effluent indicator bacteria concentrations from SCMs during the peak recreational season may have implications for both public health and watershed management and should be further evaluated by the scientific community.

5.2 Introduction

Surface waters in the United States are commonly placed on the Environmental Protection Agency's (USEPA) 303(d) list due to impairment by pathogens (indicator bacteria) (USEPA, 2008). Subsequently, indicator bacteria Total Maximum Daily Loads (TMDLs) have been established for numerous surface waters. Stormwater runoff has been identified as a contributor to indicator

bacteria pollution, with indicator bacteria concentrations in urban runoff commonly exceeding USEPA standards for surface waters (Hathaway et al. 2009, Krometis et al. 2009).

Typically, stormwater runoff mitigation involves the use of Stormwater Control Measures (SCMs – also known as Best Management Practices or "BMPs"). SCMs have been shown to effectively reduce numerous types of pollutants, yet their ability to remove indicator bacteria and pathogens is still under evaluation. Studies have indicated variable performance of SCMs for indicator bacteria from storm to storm and based on SCM type (Hathaway et al. 2009, Krometis et al. 2009, Passeport et al. 2009, Li and Davis 2009, Birch et al. 2004, Davies and Bavor 2000, Mallin et al. 2002). Evaluations of indicator bacteria removal in SCMs have typically been performed on data sets with less than 10 samples. Other than Hathaway et al. (2009), studies with more than 10 data points have collected samples at a predetermined time interval (monthly, biweekly, etc), and thus did not isolate SCM performance during storm flow.

Indicator bacteria are of particular concern in coastal areas, where human exposure can occur during recreational activities or consumption of shellfish (USEPA 2001). Such human health concerns have economic implications for the tourism and commercial fishing industries. Despite the need for microbial controls in coastal areas, few evaluations have been performed for stormwater wetlands, wet ponds, and bioretention areas in watersheds with similar characteristics to those of watersheds in the coastal Southeastern United States. In particular, limited data are present with regard to SCM removal and sequestration of enterococci, which is recommended for use as an indicator species in coastal areas and potentially has different survival characteristics than other indicator bacteria species in the environment (USEPA 2001). Only two field studies could be found in scientific literature where either a stormwater wetland or bioretention area was monitored for enterococci sequestration and removal (Davies and Bavor 2000, Jones et al. 2008)

Coastal areas in the Southeastern United States are characterized by sandy soils. This may lead to differences in SCM microbe removal efficiency. For instance, the percentage of incoming microbes attached to sediment may vary from that in clayey watersheds, as microbes predominately attach to smaller particles (Davies and Bavor 2000). Krometis et al. (2009) proposed that particle-microbe

association occurs in upland areas, further suggesting that sediment type within the watershed may influence microbial characteristics at the SCM inlet. Also, microbes may persist for longer periods in the environment when associated with particles (Sherer et al. 1992). Thus, SCMs receiving runoff with a small amount of particle associated bacteria may perform differently than those which receive high amounts of particle associated bacteria. Resuspension of captured microbe-particle colloids may also be possible in SCMs. Microbes have been shown to persist in stream and estuary sediments, where a similar environment to that found in SCMs may be present (Sherer et al. 1992, Jeng et al. 2005).Thus, scour or resuspension of sediments in SCMs during storm events may also resuspend microbes. This is specifically a concern in wet ponds and stormwater wetlands. Larger particles, such as sands, have a greater resistance to resuspension, potentially leading to reduced loss of particle associated bacteria from SCMs in sandy watersheds.

Bioretention is increasingly being used as part of Low Impact Development strategies in coastal areas. Bioretention performance for indicator bacteria has been evaluated primarily for systems constructed with media consisting of some combination of organic matter, fine particles, and sand or expanded slate fines. However, design specifications for bioretention fill media are typically focused on hydraulic efficiency (i.e., infiltration rate). Thus, it is possible that in-situ soils would be used as bioretention fill media in watersheds containing sandy soils. These potential fill soils have not been tested for indicator bacteria removal when used in bioretention designs. Although there has been some field evaluation performed on bioretention areas for indicator species removal by Hathaway et al. (2009), Li and Davis (2009), Dietz and Clausen (2005), and Passeport et al. (2009), no field evaluation has been performed on bioretention for enterococci other than a study in New England by Jones et al. (2008).

Another concern for management of surface waters is the variation observed in stormwater indicator bacteria concentrations based on season and temperature. Hathaway and Hunt (in review), Selvakumar and Borst (2006), and McCarthy et al. (2007) all showed higher indicator bacteria concentrations in stormwater runoff during warm seasons/temperatures. Such conditions coincide with peak recreational use of surface waters. In TMDL guidance provided by the USEPA, seasonal variations must be taken into account for microbial TMDLs (USEPA 2001).

Despite the understanding that indicator bacteria concentrations in stormwater runoff increase during warm periods of the year, little is known about how SCM efficiency or effluent indicator bacteria concentrations vary based on temperature and/or season. A field-monitoring study on two bioretention areas by Li and Davis (2009) observed the highest influent *Escherichia coli* (*E. coli)* and fecal coliform concentrations during the summer; however, removal efficiency could not be correlated to temperature. There are public health implications for such information, as SCM efficiency may change throughout the year. Thus, watershed plans which apply one indicator bacteria removal percentage to a given SCM (i.e., not adjusted seasonally) may misrepresent the benefit of implementing such SCMs.

The objectives of this study were to build upon the current understanding of indicator removal in SCMs by: (1) evaluating the performance of SCMs implemented in sandy, coastal watersheds for both *E. coli* and enterococci, and (2) evaluating the influence of seasonality on SCM effluent concentrations and removal of indicator bacteria.

5.3 Materials and Methods

5.3.1 Site Descriptions

The experimental sites were located in Wilmington, North Carolina (Figure 5.1). Six SCMs were evaluated, including two wet ponds, two bioretention areas, and two stormwater wetlands. Samples were collected between January 2008 and February 2010. General SCM characteristics are given in Table 5.1. Soils in the watersheds contributing to the SCMs were typically either hydrologic group A or B (sands and fine sands).

Wet Pond 1 was located in a residential medium density neighborhood with a watershed area of approximately 7 ha (Figure 5.2a). The wet pond had poor vegetative growth around its perimeter and had a sinuous pathway between the inlet and outlet. Wildlife was not readily observed around the pond, and fencing around the pond likely restricted access from domestic animals. Wet pond 2 serviced a cinema parking lot and the surrounding area (Figure 5.2b). The watershed was

approximately 15 ha. The wet pond had minimal vegetative growth along its perimeter and grass was manicured to the pond edge. Water fowl were noted at the site on occasion, but never in large quantity. Wet Pond 2 exhibited submergence at the outlet, leading to increased normal pool depth, but decreased storage depth relative to the design specifications in Table 5.1.

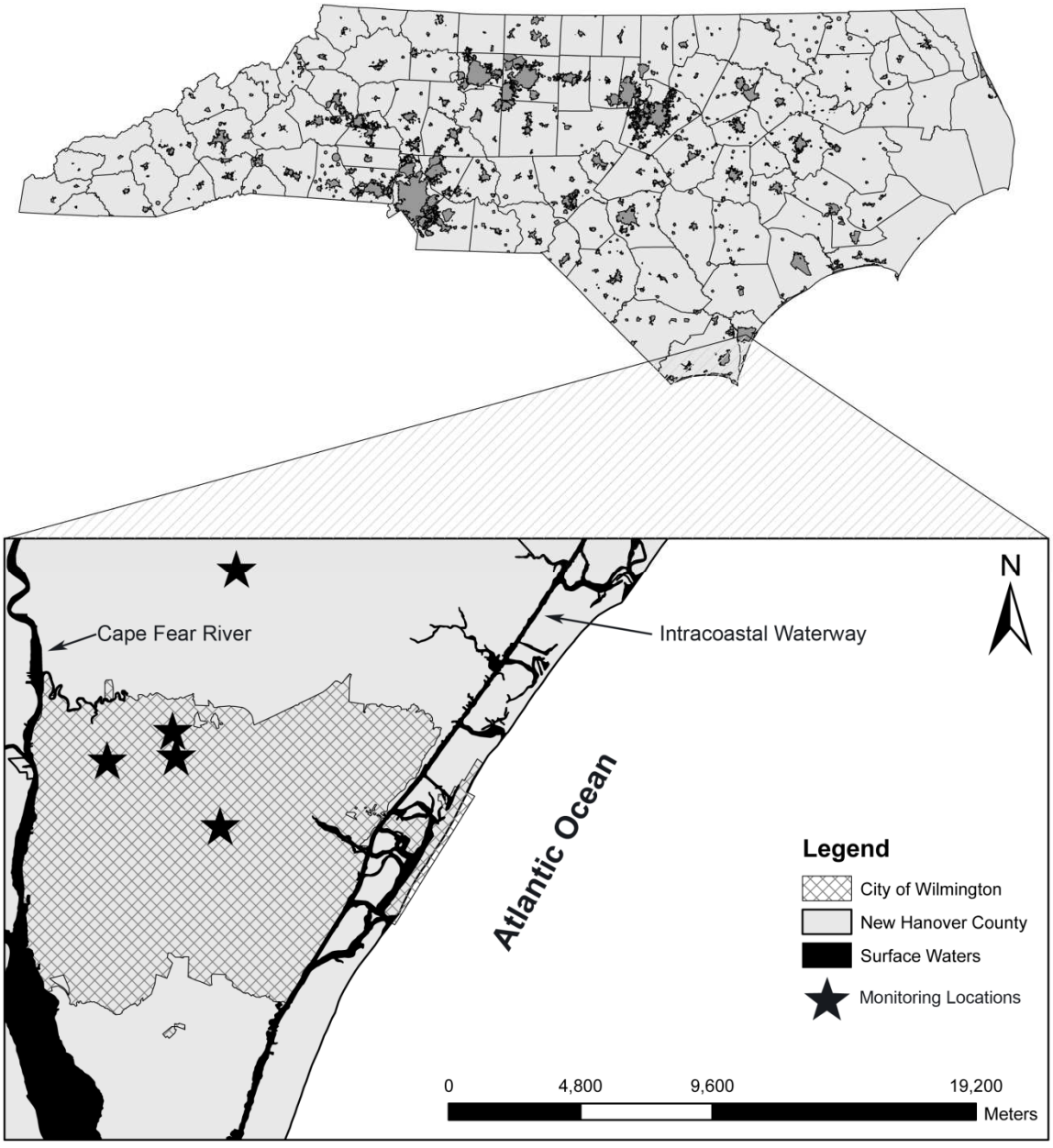

Figure 5.1: Monitoring Locations in Wilmington, NC

Table 5.1: General characteristics of Wilmington SCMs

Characteristic	Wet Pond 1	Wet Pond 2	Bioretention-D	Bioretention-S	Wetland 1	Wetland 2
Approximate Year Constructed	1999	1996	2006	2006	2005	2006
Drainage Area (ha)	7.6	14.8	0.10	0.05	12.7	2
Watershed Composition	Multi Family Residential (primarily duplex lots)	Commercial	Commercial (parking lot)	Commercial (parking lot)	Municipal (school)	Multi-family Residential
Estimated Imperviousness	45%	81%	100%	100%	20 %	42%
Primary Surrounding Soil Type (hydrologic group)[1]	Lynn Haven fine sand (B/D) and Seagate fine sand (B)	Seagate fine sand (B)	Baymeade fine sand (A)	Baymeade fine sand (A)	Leon Sand (B/D)	Baymeade fine sand (A)
Surface Area (ha)	0.18	0.59	0.006	0.006	0.1	0.09
Surface Area: Drainage Area Ratio	0.02	0.04	0.06	0.12	0.01	0.05
Storage Depth (cm)	46	52 (actual lower due backwater in effluent pipe)	28	28	31	> 15 (due to well infiltrating soils)
Estimated Average Depth (cm)	198	168	60[2]	25[2]	7	17 (typically less due to well infiltrating soils)

1. NRCS 2010 – Soil Data Mart (http://soildatamart.nrcs.usda.gov/)
2. Average depth represents soil depth for bioretention cells

(a)

(b)

(c)

(d)

(e)

Figure 5.2: Illustrations of SCMs: (a) Wet Pond 1, (b) Wet Pond 2, (c) Bioretention-D and Bioretention-S, (d) Wetland 1, and (e) Wetland 2

The two bioretention areas were located within the same parking lot which serviced a coffee shop (Figure 5.2c). A paired watershed design was sought, with each bioretention having a similar footprint, but watershed area differed due to microtopography within the parking lot. One bioretention was constructed with a soil depth of approximately 60 cm (Bioretention-D), one with a soil depth of 25 cm (Bioretention-S). All fill soil for the bioretention areas came from on site sandy soils. Each cell was constructed with a 10-cm underdrain to facilitate sample collection. It should be noted that underdrains are not typically required for bioretention areas in the sandy soils of coastal areas, thus this design differs from standard practice in the region. Runoff entered each bioretention cell as sheet flow. A small flume was installed at the pavement edge in a location presumed to be representative of the entire watershed. This allowed some pooling of runoff as it entered the bioretention cell, facilitating sampling of the inlet. The bioretention areas were covered with turf grass and had a small number of shrubs.

Wetland 1 serviced a 2-ha watershed consisting of a multi-family residential housing complex (Figure 5.2d). The wetland was constructed in sandy soils and typically had standing water only present in the deep pools. Therefore, the average depth of the system and ponding depth varied from design specifications. Wetland 2 serviced a 13-ha watershed containing a school and associated athletic fields (Figure 5.2e). Both wetlands were designed consistent with guidance by Hunt et al. (2008), including varied internal topography, emergent vegetation, and a design surface area based on capturing the water quality event for Wilmington, NC (3.8 cm). Large storms overflowed both wetlands by large weirs installed at each outlet.

5.3.2 Monitoring Methods

Short hold times and the increased man-hours and technical difficulty of using automatic samplers for microbial analyses led to the use of grab samples for SCM evaluations. This is a common methodology for sampling surface waters for indicator bacteria (USEPA 2002, Burton and Pitt 2002). All wet ponds and wetlands had one defined inlet and outlet. One sample set was collected from the inlet and outlet of each SCM for each storm event. Each sample set consisted of two sterile bottles to facilitate two bacteria analyses (*E. coli* and enterococci). Inlet samples were collected for both bioretention areas from the inlet flume mentioned previously. Outlet samples were collected from

each respective bioretention cell's underdrain. There are valid concerns over the use of grab samples, as concentrations of a given pollutant may vary during the course of the storm. However, use of grab samples was necessary in this study, and studies such as McCarthy et al. (2008) have illustrated the uncertainties present in indicator bacteria field monitoring, which potentially overshadow the negative impacts of using single grab samples to some degree.

Samples were transported to Tritest, Inc for analysis. Hold times were generally less than 6 hours. Samples were analyzed for both *E. coli* and enterococci. *E. coli* were enumerated using Colilert® and enterococci were enumerated using Enterolert®. Each methodology is based on the use of a defined substrate media (IDEXX Laboratories Inc., Westbrook, Maine). Sample dilutions were performed as needed to adequately characterize bacteria concentrations. The Limit of Detection (LOD) was typically either 2 or 10 MPN / 100 ml depending on the dilution used. The Maximum Reporting Limit (MRL) was typically 24,196 MPN / 100 ml. The MRL for *E. coli* was typically higher than that of Hathaway et al. (2009), allowing a better overall estimation of functionality. Data are analyzed herein using the values at the reporting limit without adjustment.

5.3.3 Statistical Evaluations

Statistical analyses were made to evaluate the performance of each SCM. Removal percentages (Concentration Reduction "CR") were calculated for each SCM using a similar methodology to that used to generate efficiency ratios (USEPA, 2002); however, event mean concentrations are necessary to generate efficiency ratios. This was not possible due to the use of single grab samples in this study, leading to the use of Equation 1.

$$CR = \left(1 - \frac{Geometric\ Mean\ Outlet\ Concentration}{Geometric\ Mean\ Inlet\ Concentration}\right) \times 100\% \qquad (1)$$

Microbial water quality standards are concentration based. Thus, geometric mean effluent concentrations from each SCM were compared to water quality standards for *E. coli* and

enterococci. Based on USEPA recommendations, geometric mean *E. coli* concentrations should not exceed 126 organisms / 100 ml over a 30-day period for fresh water designated as full body recreational waters (USEPA 1986). Similar recommendations exist for enterococci, whereby geometric mean concentrations should not exceed 33 organisms / 100 ml for fresh waters or 35 organisms / 100 ml for marine waters over a 30-day period (USEPA 1986).

A non-parametric Wilcoxon Signed Rank test was used to determine differences among influent and effluent concentrations. Non-parametric analyses also lessen the influence of high and low concentrations, which is important when data sets contain values below the MDL or above the MRL. These analyses were supplemented with probability plots to evaluate the performance of each SCM over the entire range of influent concentrations. Probability was calculated using Equation 2 (Burton and Pitt 2002).

$$P = \frac{(i - 0.5)}{n} \qquad (2)$$

Where: P = probability of a given observation
i = rank of observation within group n
n = number of observations within a given data set

Additional statistical analyses were performed to evaluate differences in influent and effluent concentrations based on season. Samples were categorized based on the dates considered by the North Carolina Division of Environmental Health (NCDEH) to be the “swimming season” and “non-swimming season” (NCDEH 2010). Swimming season is defined as the period between the beginning of April and the end of October. SCM functionality may hold more importance during swimming season, as water-related recreation increases. Wilcoxon Rank Sum tests were used to statistically evaluate differences in effluent concentrations between swimming and non-swimming seasons. Also, the difference in geometric mean effluent concentrations between the two seasons was calculated using Equation 3.

$$Seasonal\ Difference = \left(1 - \frac{Geometric\ Mean\ Outlet\ Concentration\ (swimming)}{Geometric\ Mean\ Outlet\ Concentration\ (non-swimming)}\right) \times 100\% \quad (3)$$

5.4 Results and Discussion

5.4.1 Summary Statistics

Between 15 and 20 storms were sampled for each SCM between January 2008 and February 2010. Summary statistics for these data are presented in Table 5.2. Samples were fairly well distributed throughout the seasons, with storm sizes ranging from 0.8 to 12.8 cm. The median storm size was 2.7 cm, suggesting the data set was somewhat shifted toward larger storm events. An analysis of historical rainfall data from Wilmington, NC, by Bean (2005) showed 70% of runoff was generated by storm events less than 2.5 cm. Because larger events may create more scour and decrease detention time in SCMs, these data are likely conservative estimates of SCM function. There is some concern that large storms may dilute indicator species, resulting in decreased influent concentrations; however, McCarthy et al. (2007) and Hathaway and Hunt (in review) showed no significant correlation between indicator bacteria concentrations in urban stormwater runoff and storm size.

The geometric mean of the influent *E. coli* samples was between 130 and 2483 MPN / 100 ml. This range was similar to that reported for *E. coli* concentrations entering SCMs in Charlotte, NC, by Hathaway et al. (2009) and influent concentrations for one of two wet ponds studied in Durham, NC, by Krometis et al. (2009). Geometric mean enterococci concentrations ranged from 274 to 2356, slightly higher than influent concentrations for SCMs studied by Jones et al. (2008), but lower than values reported for influent concentrations to wet ponds studied by Krometis et al. (2009).

Table 5.2: Summary statistics for monitored storm events

Location	Number of Samples	Statistic	*E. coli*		enterococci	
			MPN / 100 ml		MPN / 100 ml	
			inlet	outlet	inlet	outlet
Wet Pond 1	15	geometric mean	2483	62	2356	237
		median	2851	40	2599	168
		maximum	24196	19863	24196	24196
		minimum	255	2	278	2
		standard deviation	6555	5082	8111	6491
Wet Pond 2	18	geometric mean	1273	60	274	37
		median	2489	34	179	20
		maximum	81640	3466	24196	1633
		minimum	10	2	2	2
		standard deviation	19639	843	6218	563
Bioretention-D	20	geometric mean	130	39	375	39
		median	122	10	440	42
		maximum	7701	8164	4839	1454
		minimum	2	2	30	2
		standard deviation	2106	1959	1355	323
Bioretention-S	20	geometric mean	130	284	375	378
		median	122	714	440	358
		maximum	7701	19863	4839	4839
		minimum	2	2	30	20
		standard deviation	2106	5632	1355	1536
Wetland 1	18	geometric mean	834	826	1018	316
		median	741	1167	1097	309
		maximum	14136	36540	24196	29090
		minimum	75	17	61	2
		standard deviation	3833	9870	6135	8437
Wetland 2	18*	geometric mean	425	503	866	510
		median	554	386	842	690
		maximum	9804	24196	24196	24196
		minimum	10	6	140	10
		standard deviation	2516	6335	5428	5827

* 19 enterococci samples collected for Wetland 2

5.4.2 Concentration Reduction

Concentration reductions for each SCM are documented in Tables 5.3 and 5.4. Highest *E. coli* reductions were observed for the wet ponds, which also had the highest influent concentrations. Bioretention-D also performed well with a concentration reduction of 70%. Poor performance was

noted for Bioretention-S and the two wetlands for *E. coli*, although both wetlands had fair removal of enterococci. For each SCM, removal performance was variable from storm to storm. Individual event concentration reductions varied from greater than 90% to an *addition* of both *E. coli* and enterococci for most SCMs. Similar inter-event variations in SCM performance for indicator bacteria were showed for bioretention areas by Li and Davis (2009) and for a stormwater wetland by Birch et al. (2004).

Table 5.3: *E. coli* concentration reductions for Wilmington SCMs

SCM Type	*E. coli* Concentrations (MPN/100ml)		
	Geometric Mean Influent	Geometric Mean Effluent	Concentration Reduction (%)
Wet Pond 1	2483	62	98
Wet Pond 2	1273	60	95
Bioretention-D	130	39	70
Bioretention-S	130	284	-119
Wetland 1	834	826	1
Wetland 2	425	503	-18

Table 5.4: Enterococci concentration reductions for Wilmington SCMs

SCM Type	Enterococci Concentrations (MPN/100ml)		
	Geometric Mean Influent	Geometric Mean Effluent	Concentration Reduction
Wet Pond 1	2356	237	90
Wet Pond 2	274	37	87
Bioretention-D	375	39	89
Bioretention-S	375	378	-1
Wetland 1	1018	316	69
Wetland 2	866	510	41

Results of a Wilcoxon Signed Rank analysis are shown in Table 5.5. Only the two wet ponds significantly reduced *E. coli* ($p < 0.05$), while both wet ponds and Bioretention-D significantly reduced enterococci. Significant relationships can be difficult to find in microbial data sets given the inter-storm performance variability noted in these data and in other studies. Statistical analyses generally support the concentration reductions in Tables 5.3 and 5.4, as wet ponds and Bioretention-D were found to perform well.

Table 5.5: Results of Wilcoxon Signed Rank Analysis

Location	*E.coli*		enterococci	
	p - value	significant difference?	p - value	significant difference?
Wet Pond 1	0.0002	**yes**	0.0134	**yes**
Wet Pond 2	0.001	**yes**	0.001	**yes**
Bioretention-D	0.1926	no	0.0001	**yes**
Bioretention-S	0.0808	no	0.5459	no
Wetland 1	0.6095	no	0.1187	no
Wetland 2	0.6322	no	1	no

Wet ponds have shown varied levels of treatment for indicator bacteria. A study of two wet ponds in Durham, NC, by Krometis et al. (2009) yielded different results. One pond showed poor performance with geometric mean concentration reductions of -41%, 0%, and -108% for fecal coliform, *E. coli,* and enterococci, respectively. The second pond showed modest removal, reducing geometric mean concentrations by 31%, 48%, and 36% for fecal coliform, *E.coli*, and enterococci, respectively. A study by Davies and Bavor (2000) on a wet pond near Sydney, Australia, showed similarly poor performance with fecal coliform and enterococci removal efficiencies of -2.5% and 23%, respectively. In a study by Mallin et al. (2002), 2 of 3 wet ponds in Wilmington, NC, removed fecal coliform with an efficiency higher than 50%, with the third wetland showing negative removal. Positive removal of indicator bacteria was also reported by Hathaway et al. (2009) for a wet pond in Charlotte, NC, with concentration reductions of 70% and 46% for fecal coliform and *E. coli*, respectively. Thus, from a removal efficiency metric, large variations in performance have been noted for wet ponds. Generally, performance for the wet ponds in Wilmington, NC, studied herein was good compared to studies in literature, and compared similarly to 1 of 3 wet ponds studied by Mallin et al. (2002) in Wilmington, NC, where a fecal coliform concentration reduction of over 85% was found. It should be noted that the other two ponds studied by Mallin et al. (2002) had low influent fecal coliform concentrations, 97 organisms / 100 ml and 74 organisms / 100 ml, potentially influencing microbial removal efficiency. Relatively poor performing wet ponds studied by Krometis et al. (2009) and Davies and Bavor (2002) potentially had different influent microbial particle association characteristics than those in Wilmington, NC, as finer soil types were likely present in their contributing watersheds. The soil types in the watersheds supplying runoff to the Wilmington, NC, SCMs were predominately fine sand. This may have implications for the amount of bacteria

attached to particles at the inlet and the likelihood of resuspension of captured sediments (and associated bacteria) during subsequent events. Further, particle associated microbes have been shown to exhibit higher resistance to environmental conditions that otherwise cause their die-off (Sherer et al. 2002). Also, a high water table, characteristic of coastal areas in the Southeastern United States, may have resulted in dilution due to groundwater intrusion into the ponds. One of the two wetlands (Wetland 1) also intersected the groundwater table, but an improved performance was not evident.

Stormwater wetland indicator bacteria sequestration and removal has not been studied at length in peer-reviewed literature. A study by Birch et al. (2004) showed mean fecal coliform removal of 76%. Davies and Bavor (2002) reported removal efficiencies of 79% and 85% for fecal coliform and *E. coli*, respectively. However, results for two wetlands in Charlotte, NC, studied by Hathaway et al. (2009) were variable. One wetland exhibited fecal coliform and *E. coli* removal of 98% and 96%, respectively, while the other showed fecal coliform and *E. coli* removal of 56% and 33%, respectively. It should be noted that Hathaway et al. (2009) attributed high removal of indicator bacteria to a lack of vegetation in one of the wetlands, but vegetation deficiency is not a desirable attribute for stormwater wetlands. The results of studies in scientific literature generally indicate fair performance of stormwater wetlands for indicator bacteria. However, data from this research suggests poor performance of stormwater wetlands for *E. coli* removal, and modest performance for enterococci.

Differences in microbial removal efficiency between stormwater wetlands and wet ponds are not well established. A comparison of stormwater wetland and wet pond performance by Davies and Bavor (2002) indicated that wetlands may be more adept at indicator bacteria removal. However, a review of data from the International Stormwater BMP Database by USEPA (2003) suggested that wet ponds performed better for fecal coliform than wetlands and that data are less variable from site to site. For the SCMs studied in Wilmington, NC, as part of this study, wet ponds appeared superior for removal of indicator bacteria. Numerous factors are likely associated with removal of indicator bacteria in stormwater wetlands and wet ponds, including predation, settling of particle associated microbes, and potential resuspension of captured particle associated microbes due to

internal SCM hydrodynamics. Thus, numerous variables are present in stormwater wetlands and wet ponds which may explain variations in performance. Further research is needed to determine factors which contribute to the performance of these SCMs regarding microbe die-off.

Few field evaluations of indicator bacteria removal have been performed for bioretention, particularly for enterococci. Studies by Hathaway et al. (2009) on a bioretention area in Charlotte, NC, and Passeport et al. (2009) on two bioretention cells in Graham, NC, indicated high fecal coliform concentration reductions, with all three cells having concentration reductions above 85%. Hathaway et al. (2009) also reported a 92% *E. coli* concentration reduction for the bioretention area in Charlotte, NC. Jones et al. (2008) examined enterococci removal from a bioretention area in New Hampshire showing a concentration reduction of over 90%. Conversely, evaluations by Li and Davis (2009) on two bioretention areas in Silver Spring and College Park, MD, showed relatively poor performance for *E. coli* (median removal of 0% and 57%, respectively) and fecal coliform (median removal of 50% and 0%, respectively). Likewise, there was a substantial difference in functionality between the two bioretention areas studied in Wilmington, NC. The differing depth of media, nominally 60 cm for Bioretention-D and 25 cm for Bioretention-S, appeared to result in varied performance. Further investigation is planned to explore possible explanations for the difference in performance between cells. Potential causes are differences in organic content of the soils in the two cells, differences in soil moisture, differences in soil temperature, and differences in hydraulic function of the two systems (leading to differences in microbial sorption). It should be noted that both bioretention areas were constructed using native soils as fill media. Native soils were generally fine sands (NRCS 2010), which may lead to reduced effectiveness. Small clay particles have a greater ability than sand to facilitate sorption of microbes (Mankin et al. 2007).

5.4.3 Influent and Effluent Probability Plots

Probability plots allowed greater examination of influent and effluent indicator bacteria relationships for each SCM. Probability plots for *E. coli* are presented in Figures 5.3a-5.3e. Probability plots for enterococci are presented in Figures 5.4a-5.4e. These plots generally support performance observations made previously. Separation between influent and effluent probability curves are particularly noted for both wet ponds for *E. coli* and enterococci and Bioretention-D for

enterococci. Some consistent separation between influent and effluent *E. coli* probability curves is noted for Bioretention-D; however, the separation is moderate in comparison to that exhibited in its enterococci probability plot. Wetland 1 also appears to function fairly well for enterococci based on the probability plots, supporting the moderate removal efficiency noted in Table 5.4. Probability plots for Bioretention-S for both indicator bacteria and the stormwater wetlands for *E. coli* show a lack of distinction between influent and effluent probability curves, indicating inconsistent, poor performance over the course of the study.

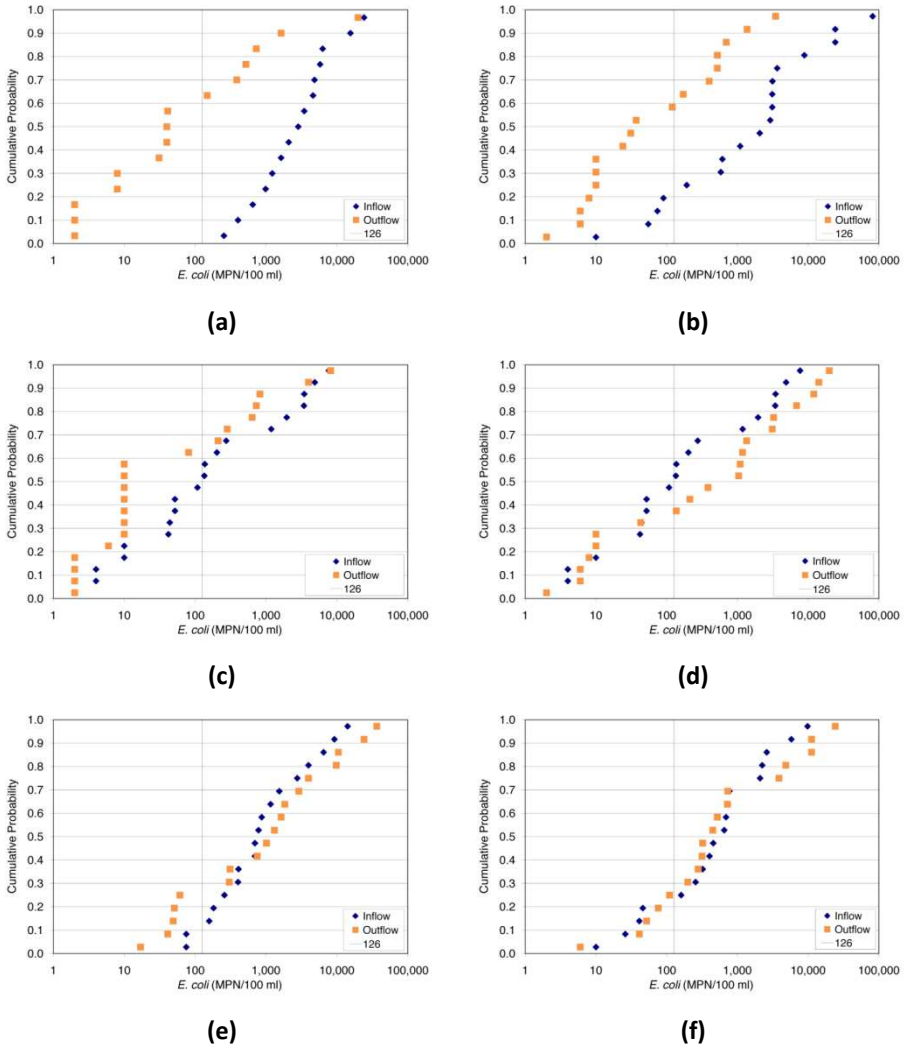

Figure 5.3: *E. coli* probability plots for (a) Wet Pond 1, (b) Wet Pond 2, (c) Bioretention-D, (d) Bioretention-S, (e) Wetland 1, and (f) Wetland 2

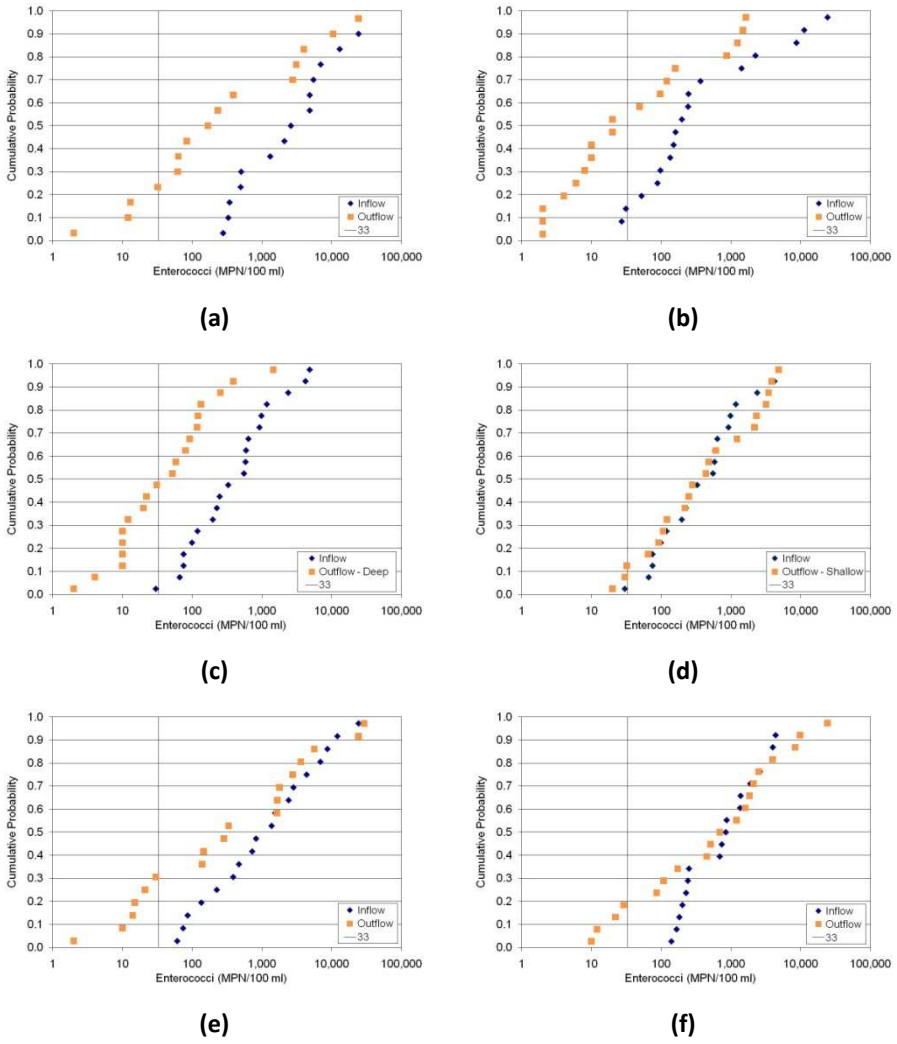

Figure 5.4: Enterococci probability plots for (a) Wet Pond 1, (b) Wet Pond 2, (c) Bioretention-D, (d) Bioretention-S, (e) Wetland 1, and (f) Wetland 2

5.4.4 Analysis of Effluent Concentrations

Microbial contamination is regulated by target concentrations established by the USEPA (1986). For fresh waters regulated for full body contact, the geometric mean over a 30-day period cannot exceed 126 organisms / 100 ml for *E. coli* or 33 organisms / 100 ml for enterococci. For marine waters regulated for full body contact, the geometric mean over a 30-day period cannot exceed 35 organisms / 100 ml for enterococci. Thus, effluent SCM concentrations can be compared to these values to determine how they will affect concentrations in receiving waters. Obviously, mass

balances would be required to evaluate the full impact of these practices on targeted watersheds. Median effluent indicator bacteria concentrations are shown in Tables 5.6 and 5.7.

Table 5.6: Median effluent *E. coli* concentrations

SCM Type	*E. coli* Concentrations (MPN/100ml)			
	Geometric Mean Influent	Geometric Mean Effluent	Number of effluent samples less than 126 MPN / 100 ml	number of effluent samples less than 126 MPN / 100 ml (percentage)
Wet Pond 1	2483	62	9	9 of 15 (60%)
Wet Pond 2	1273	60	11	11 of 18 (61%)
Bioretention-D	130	39	13	13 of 20 (65%)
Bioretention-S	130	284	7	7 of 20 (35%)
Wetland 1	834	826	5	5 of 18 (28%)
Wetland 2	425	503	5	5 of 18 (28%)

Table 5.7: Median effluent enterococci concentrations

SCM Type	Enterococci Concentrations (MPN/100ml)			
	Geometric Mean Influent	Geometric Mean Effluent	Number of effluent samples less than 33 MPN / 100 ml	Number of effluent samples less than 33 MPN / 100 ml (percentage)
Wet Pond 1	2356	237	4	4 of 15 (27%)
Wet Pond 2	274	37	10	10 of 18 (56%)
Bioretention-D	375	39	10	10 of 20 (50%)
Bioretention-S	375	378	3	3 of 20 (15%)
Wetland 1	1018	316	6	6 of 18 (33%)
Wetland 2	866	510	4	4 of 18 (22%)

SCMs that provided good removal of indicator bacteria (Tables 5.3 and 5.4) also had low geometric mean effluent concentrations. Median effluent *E. coli* concentrations were below USEPA target concentrations for Wet Pond 1, Wet Pond 2, and Bioretention-D. For enterococci, no SCM had median effluent concentrations below USEPA targeted values, although Wet Pond 2 and Bioretention-D approached targeted values.

No SCM consistently provided *E. coli* or enterococci concentrations lower than USEPA targeted values. Wet Pond 2 and Bioretention-D provided the highest percentage of effluent *E. coli* and enterococci samples below the target value, while Wet Pond 1 had a high percentage of storms below only the *E. coli* target value. Bioretention-S and the two stormwater wetlands did not typically have effluent concentrations below the USEPA target values.

These results suggest that although positive reductions of indicator bacteria can be observed in SCMs, even those which perform well may not consistently produce concentrations below USEPA target values for surface waters. Similar observations were made by Hathaway et al. (2009). This is important in evaluating the effectiveness of watershed restoration activities. To reliably reduce indicator bacteria loadings to surface waters, SCMs must reduce runoff volume. SCMs may not consistently contribute to watershed restoration simply due to concentration reductions. To this end, a SCM like bioretention that has been repeatedly shown to reduce outflow volumes (Hunt et al. 2006, Li et al. 2009) holds the most promise.

Estimations of non-exceedance probabilities were generated using probability plots. A regression line was fit to the outlet data and the non-exceedance probability was estimated as the probability where the regression line crossed the USEPA targeted surface water concentration for *E. coli* and enterococci, respectively. This allowed some estimation of the probability a given SCM's effluent concentration will not exceed USEPA targeted surface water concentrations. Approximate non-exceedance probabilities are presented in Table 5.8. Generally, there is a higher probability of exceeding the enterococci target concentration, with non-exceedance probabilities being lower than 50% for all SCMs. Non-exceedance probabilities for *E. coli* were higher than 50% for Wet Pond 1, Wet Pond 2, and Bioretention-D. The Bioretention-D non-exceedance probability for *E. coli* approached that observed for two bioretention areas evaluated by Li and Davis (2009), where non-exceedance probabilities for *E. coli* were estimated as > 65% and >75%.

Table 5.8: USEPA targeted concentration non-exceedance probabilities

SCM Type	Approximate Non-exceedance Probability (%)	
	E. coli	enterococci
Wet Pond 1	57	29
Wet Pond 2	60	49
Bioretention-D	63	47
Bioretention-S	43	9
Wetland 1	26	26
Wetland 2	32	16

5.4.5 Seasonal Impacts on SCM Effluent Concentrations

The public health impacts of urban stormwater runoff are of particular interest during periods of the year when water related recreational activities are most common. Studies such as Hathaway and Hunt (in review), Selvakumar and Borst (2006), and Line et al. (2008) suggest indicator bacteria concentrations in stormwater runoff may increase with warmer seasons/temperatures. Data were separated into swimming and non-swimming periods based on dates used as guidelines for compliance sampling by the NCDEH (2010). The non-swimming period is from November to the end of March, when average daily temperatures are lowest. For each SCM, both swimming and non-swimming seasons were represented by at least 5 samples (Table 5.9). The influent and effluent concentrations of *E. coli* and enterococci for each SCM are illustrated in Figures 5.5 and 5.6 for the two periods (swimming and non-swimming).

Table 5.9: Number of swimming and non-swimming samples for each SCM for both indicator bacteria

Location	Number of Samples	
	swimming	non-swimming
Wet Pond 1	10	5
Wet Pond 2	10	8
Bioretention-D	10	10
Bioretention-S	10	10
Wetland 1	10	8
Wetland 2	10	8*

* 9 non-swimming enterococci samples

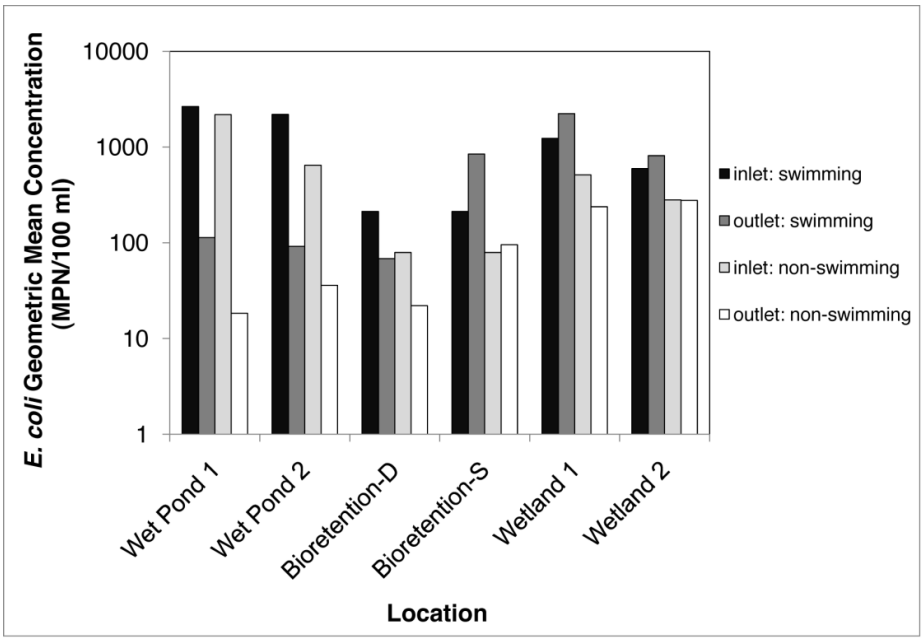

Figure 5.5: Geometric mean influent and effluent *E. coli* concentrations for swimming and non-swimming seasons for each SCM

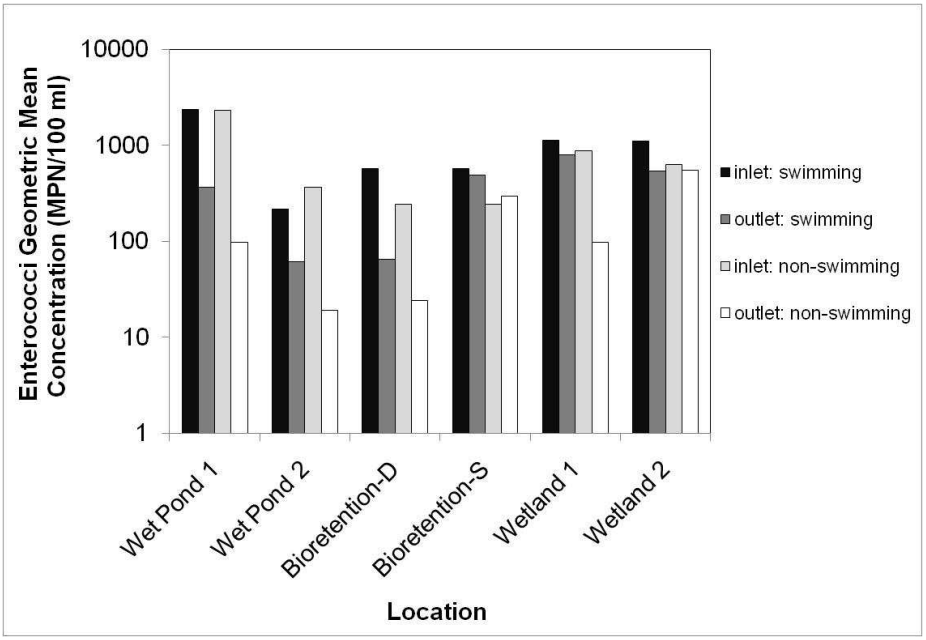

Figure 5.6: Geometric mean influent and effluent enterococci concentrations for swimming and non-swimming seasons for each SCM

Geometric mean concentrations of *E. coli* were higher during the swimming season for both the inlet and outlet of each SCM. For enterococci, this was also true for the majority of SCMs. Geometric mean inlet enterococci concentrations were found to be higher during the non-swimming season for Wet Pond 2, and geometric mean outlet enterococci concentrations were found to be higher during the non-swimming season for Wetland 2. Table 5.10 shows the difference in geometric mean indicator bacteria concentrations between the swimming and non-swimming seasons as calculated by equation 3. Effluent concentrations decreased by more than 60% for both indicator bacteria in all SCMs (other than enterococci in Bioretention-S and Wetland 2).

Table 5.10: Analysis of seasonal differences in effluent concentrations

Location	Effluent concentration reduction - swimming to non-swimming (%)	
	E.coli	enterococci
Wet Pond 1	84	73
Wet Pond 2	61	69
Bioretention-D	68	63
Bioretention-S	89	40
Wetland 1	89	88
Wetland 2	66	-2

Despite apparent differences between swimming and non-swimming periods, Wilcoxon Rank Sum tests showed no statistically significant differences between inlet and outlet concentrations for the two seasons ($p < 0.05$). It is possible the high variability common in microbiological data resulted in statistically insignificant results; further, a relatively low number of non-swimming samples (5) was available for Wet Pond 1. Nonetheless, these data suggest effluent indicator bacteria concentrations may vary seasonally for SCMs. A study on two bioretention areas by Li and Davis (2009) identified summer as the season when the highest influent concentrations of *E. coli* and fecal coliforms were found for each system; however, removal performance could not be correlated to temperature. Similar observations of higher effluent enterococci concentrations during the summer and early fall were made by Jones et al. (2008) on a wet pond in New Hampshire.

Temporal changes in both influent and effluent indicator bacteria concentrations could also lead to differences in SCM removal efficiency throughout the year. Table 5.11 shows indicator bacteria concentration reductions in SCMs during the swimming and non-swimming seasons. Concentration reductions are typically higher during the non-swimming season, although systems which performed well overall (Wet Pond 1, Wet Pond 2, and Bioretention-D) provided relatively high concentration reductions throughout the year. Nonetheless, these data suggest SCM effectiveness for indicator bacteria may vary throughout the year, but more data are needed to strengthen this postulation. This represents a future research need in the stormwater management field, and could have implications for both public health and watershed management.

Table 5.11: Indicator bacteria concentration reductions in SCMs during swimming and non-swimming seasons

Location	*E.coli*		Enterococci	
	Concentration Reduction - swimming (%)	Concentration Reduction - non-swimming (%)	Concentration Reduction - swimming (%)	Concentration Reduction - non-swimming (%)
Wet Pond 1	96	99	84	96
Wet Pond 2	96	94	71	95
Bioretention-D	68	72	89	90
Bioretention-S	-297	-21	15	-20
Wetland 1	-81	53	30	89
Wetland 2	-36	1	52	14

5.5 Conclusions

Six Stormwater Control Measures were evaluated for *E. coli* and enterococci removal over 15 to 20 storm events in Wilmington, NC. Both wet ponds and the deep bioretention cell were effective at removing both *E. coli* and enterococci, with concentration reductions exceeding 70% for both indicator bacteria in each SCM. However, the shallow bioretention cell and both stormwater wetlands did not perform well in comparison, particularly for *E. coli*. These data suggest some SCMs can export indicator bacteria, as two of the six SCMs showed negative removal of *E. coli*. Similar results have been seen in such studies at Krometis et al. (2009), Li and Davis (2009), Jones et al. (2008), and Hathaway et al. (2009). These results are not illogical, as indicator bacteria have been shown to persist in sediments of streams and estuaries (Sherer et al. 1992, Jeng et al. 2005). Further,

studies by Davies and Bavor (2000) on wet pond sediments indicated the persistence of indicator bacteria even after 28 days. SCMs may also attract wildlife, leading to direct addition of indicator bacteria into the system through defecation.

These data have some similarity to other studies which evaluated microbial reductions in SCMs; however, some differences in performance may occur based on geophysical region. This is possibly due to differences in particle-microbe interactions in sandy watersheds and/or dilution from the water table. In particular, wet ponds evaluated in this study and one of three evaluated by Mallin et al. (2002) performed well in comparison to wet ponds studied in clayey watersheds by Krometis et al. (2009) and Davies and Bavor (2000). Also, further study is needed to determine how soil type and design configuration affect indicator bacteria removal in bioretention areas. Despite a larger watershed, Bioretention-D performed well in comparison to Bioretention-S. Both the depth and type of fill media likely influence the ability of Bioretention-S to sequester bacteria.

SCMs which performed well in Wilmington, NC, showed promise in meeting USEPA target *E. coli* concentrations for surface waters. Both wet ponds and Bioretention-D had geometric mean effluent *E. coli* concentrations lower than USEPA target values. Enterococci target values were not achieved by any SCM; however, both Wet Pond 2 and Bioretention-D had geometric mean effluent concentrations which approached target concentrations. Although this creates some concern as to the benefit of SCMs in watersheds impacted by microbial pollution, a SCM's contribution to watershed restoration cannot be evaluated based on concentration reduction alone. Reductions in indicator bacteria mass entering surface waters may be achieved through such mechanisms as infiltration. Evaluation of the impacts of infiltration on groundwater microbial quality represents another need within the field of stormwater management, particularly as infiltration-based SCMs become increasingly implemented.

SCM effluent indicator bacteria concentrations appear to vary throughout the year. Specifically, effluent concentrations may elevate during the swimming season from April to October. Further research is needed to verify this observation due to the variability in these data and lack of statistically significant results. Elevated concentrations during the period of the year when water

related recreational activities are most frequent causes some concern in regard to public health. Understanding these changes is important in determining how to manage watersheds for indicator bacteria. TMDLs for microbial pollution are required to account for seasonal variability. Seasonal variability has been shown for indicator bacteria in urban stormwater runoff in such studies at Selvakumar and Borst (2006) and Hathaway and Hunt (in review). Further research is needed to determine if there are also seasonal differences in SCM indicator bacteria sequestration. This could be included in watershed TMDLs, as varied load reductions could be applied to SCMs based on season.

Despite a recent increase in the number of studies evaluating indicator bacteria performance of SCMs, data are variable. Further, there are limited data in regard to SCM performance for enterococci, the USEPA recommended indicator bacteria for marine environments. A relatively limited amount of scientific literature has shown differences in performance of SCMs for fecal coliform, *E. coli*, and enterococci. The data presented herein have added to the limited scientific knowledgebase for SCM performance for enterococci. Removal of enterococci was found to vary substantially from removal of *E. coli* for a number of the six SCMs evaluated. Differences in removal of *E. coli* and enterococci were not consistent, as some SCMs performed better for *E. coli* and others for enterococci. However, effluent enterococci concentrations did not approach USEPA target concentrations even in SCMs which had effluent *E. coli* concentrations less than USEPA targeted concentrations. Further, non-exceedance probabilities were lower for enterococci for all SCMs other than Wetland 1. It is unknown if these differences are due to variations in the magnitude of influent and effluent microbe populations, or due to differences in indicator persistence. For instance, enterococci are typically regarded as being more resistant to environmental conditions (USEPA 2001). At this time, it does not appear that similar performance can be assumed for a given SCM for all indicator bacteria.

Perhaps the most important need in examining indicator bacteria removal in SCMs is an understanding of mechanisms which control indicator bacteria persistence and sequestration. Such understanding will: (1) help determine which SCMs should be used in watersheds impacted by microbial pollution, (2) allow a greater understanding of the public health implications of indicator

bacteria persistence in SCMs, (3) explain the variability noted in this study and others with regard to SCM removal efficiency of indicator bacteria, and (4) potentially lead to design modifications which can be used for SCMs in an effort to enhance removal of indicator bacteria.

5.6 Acknowledgements

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5.7 References

Bean, E.Z. (2005). "A field study to evaluate permeable pavement surface infiltration rates, runoff quantity, runoff quality, and exfiltrate quality." Master of Science Thesis. Raleigh, NC: Department of Biological and Agricultural Engineering, NC State University.

Birch, G.F., C. Matthai, M.S. Fazeli, and J.Y. Suh. (2004). "Efficiency of a constructed wetland in removing contaminants from stormwater." *Wetlands*, 24(2): 459-466.

Burton, G.A. and R.E. Pitt. (2002). "Stormwater Effects Handbook: A Toolbox for Watershed Managers, Scientists, and Engineers." CRC Press, LLC, Boca Raton, Fl.

Davies, C.M. and H.J. Bavor. (2000). "The Fate of Stormwater-Associated Bacteria in Constructed Wetland and Water Pollution Control Pond Systems." *Journal of Applied Microbiology*. 89: 349-360.

Dietz, M.E., and J.C. Clausen. (2005). "A field evaluation of rain garden flow and pollutant treatment." *Water, Air, and Soil Pollution*, 167: 123-138.

Hathaway, J.M., and W.F Hunt. (in review). "Statistical evaluation of factors affecting indicator bacteria in urban stormwater runoff." *Journal of Environmental Engineering*.

Hathaway, J.M., W. F. Hunt, and S. Jadlocki. (2009). “Indicator bacteria removal in storm-water best management practices in Charlotte, North Carolina.” *Journal of Environmental Engineering*, 135(12): 1275 – 1285.

Hunt, W.F., A.R. Jarrett, J.T. Smith, L.J. Sharkey. (2006). “Evaluating bioretention hydrology and nutrient removal at three field sites in North Carolina.” *Journal of Irrigation and Drainage Engineering*, 132(6): 600 - 608.

Hunt, W.F., M.R. Burchell, J.D. Wright, and K.L. Bass. (2007). “Stormwater wetland design update: zones, vegetation, soil, and outlet guidance.” AGW-588-12. *North Carolina Cooperative Extension*, Raleigh, N.C.

Jeng, H.C., A.J. England, and H.B. Bradford. (2005). “Indicator organisms associated with stormwater suspended particles and estuarine sediment.” *Journal of Environmental Science and Health*, 40: 779-791.

Jones, S.H., R. Roseen, R. A. Wildey, J. Maimes, J. Houle, T. Ballestero. (2008). “Enterococci populations in conventional, manufactured, and LID stormwater treatment systems.” *Proc., World Environmental and Water Resources Congress*, ASCE, Reston, VA.

Krometis, L.H, P.N. Drummey, G.W. Characklis, and M.D. Sobsey. (2009). “Impact of microbial partitioning on wet retention pond effectiveness.” *Journal of Environmental Engineering*, 135(9): 758-767.

Li, H., and A.P. Davis. (2009). “Water quality improvement through reductions of pollutant loads using bioretention.” *Journal of Environmental Engineering*, 135(8): 567-576.

Li, H., L.J. Sharkey, W.F. Hunt, A.P. Davis. (2009). “Mitigation of impervious surface hydrology using bioretention in North Carolina and Maryland.” *Journal of Hydrologic Engineering*, 14(4): 407-415.

Line, D.E., N.M. White, W.W. Kirby-Smith, and J.D. Potts. (2008). “Fecal coliform export from four coastal North Carolina areas.” *Journal of the American Water Resources Association*, 44(3): 606-617.

Mallin, M.A., S.H. Ensign, T.L. Wheeler, and D.B. Mayes. (2002). “Pollutant removal efficacy of three wet detention ponds.” *Journal of Environmental Quality*, 31: 654-660.

Mankin, K.R., L. Wang, S.L. Hutchinson G.L. Marchin. (2007). “*Escherichia coli* sorption to sand and silt load soil.” *Transactions of the ASABE*, 50(4): 1159-1165.

McCarthy, D.T., V.G. Mitchell, A. Deletic, and C. Diaper. (2007). “*Escherichia coli* in urban stormwater: explaining their variability.” *Water Science and Technology*, 56(11), 27-34.

McCarthy, D.T., A. Deletic, V.G. Mitchell, T.D. Fletcher, C. Diaper. (2008). “Uncertainties in stormwater *E. coli* levels.” *Water Research*, 42: 1812-1824.

Natural Resources Conservation Service (NRCS). (2010). “Soil data mart.” http://soildatamart.nrcs.usda.gov/ (March 25, 2010).

North Carolina Division of Environmental Health (NCDEH). (2010). “N.C. Recreational Water Quality.” http://www.deh.enr.state.nc.us/shellfish/Water_Monitoring/RWQweb/home.htm (March 25, 2010).

Passeport, E., W.F. Hunt, D.E. Line, R.A. Smith, and R.A. Brown. (2009). “Field study of the ability of two grassed bioretention cells to reduce storm-water runoff pollution.” *Journal of Irrigation and Drainage Engineering*, 135(4): 505 – 510.

Selvakumar, A., and M. Borst. (2006). Variation of microorganism concentrations in urban stormwater runoff with land use and seasons. *Journal of Water and Health*, 4(1): 109-124.

Sherer, B.M., J.R. Miner, J.A. Moore, J.C. Buckhouse. (1992). “Indicator bacteria survival in stream sediments.” *Journal of Environmental Quality*, 21: 591-595.

USEPA. (1986). “Ambient water quality criteria for bacteria – 1986.” *EPA 440/5-84-002*. Office of Water, Washington, DC.

USEPA. (2001). “Protocol for developing pathogen TMDLs.” *EPA-841-R-00-002*. Office of Water, Washington, DC.

USEPA. (2002). “Urban stormwater BMP performance monitoring.” *EPA-821-B-02-001*. Office of Water, Washington, DC.

USEPA. (2003). “Bacterial water quality standards for recreational waters: status report.” *EPA-823-R-03-008*. Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA). (2008). “Water quality assessment and total maximum daily loads information (ATTAINS). “ http://www.epa.gov/waters/ir/index.html (November 29, 2008).

6 Analysis of Factors Influencing Bioretention Performance for Indicator Bacteria in Wilmington, NC

6.1 Abstract

Although bioretention has been shown to remove or sequester a wide range of pollutants, relatively little study has been performed to evaluate its ability to sequester indicator bacteria. Two bioretention areas in Wilmington, NC, were studied in a paired-watershed experimental design. The primary difference in the design of the two systems was soil depth. One bioretention cell was constructed with 25 cm of fill soil (Bioretention-S) and one with 60 cm of fill soil (Bioretention-D). The systems were found to perform differently for indicator bacteria based on multiple performance evaluation metrics. Bioretention-D showed concentration reductions of 70% and 89% for *E. coli* and enterococci, respectively. Effluent concentrations from Bioretention-D compared well to EPA target values and other studies in literature. Conversely, Bioretention-S showed concentration "reductions" of -119% and -102% for *E. coli* and enterococci, respectively. Effluent concentrations from Bioretention-S were substantially higher than USEPA target values and other studies in literature. Multiple factors were evaluated to determine the cause of performance differences between the two cells. Soil depth was identified as the most important factor. The 25 cm of fill soil in Bioretention-S exhibited poorer runoff detention and theoretically resulted in higher soil water flux and decreased contact time relative to Bioretention-D. These differences seemingly led to diminished indicator bacteria sequestration. The results of this study suggest soil depth is an important design parameter for bioretention which should be carefully selected. Further, minimum soil depths appear to exist, below which decreased sequestration of indicator bacteria may be experienced.

6.2 Introduction

Low Impact Development (LID) is increasingly utilized as a technique to mitigate the impact of stormwater runoff on surface waters (USEPA 2000). As part of LID, infiltration based SCMs (Stormwater Control Measures – also known as Best Management Practices, "BMPs," Water Sensitive Urban Designs, and "WSUDs") are implemented to facilitate stormwater treatment, groundwater recharge, and stormwater volume and peak reductions. One commonly utilized SCM as

part of LID is bioretention (also known as biofiltration, or bio-infiltration when underdrains are not employed).

Bioretention has been shown effective at reducing runoff volumes, peak flows, and numerous pollutants ranging from nutrients to metals (Hunt et al. 2006, Dietz and Clausen 2005, Davis et al. 2006, Davis et al. 2009, Roseen et al. 2006). However, until recently, little was known regarding bioretention sequestration of indicator bacteria. Indicator bacteria denote contamination from fecal matter and thus the possible presence of pathogens. In a review of bioretention literature and future needs, Davis et al. (2009) identified research on bioretention removal of pathogenic bacteria as a need for the stormwater management community. Indicator bacteria are a common source of impairment in surface waters in North America, Europe, Australia, and elsewhere. In the United States, there are more Total Maximum Daily Loads (TMDLs) in place for indicator bacteria than any other pollutant (USEPA 2010). Stormwater runoff from urban watersheds has been shown to have substantial concentrations of indicator bacteria (Selvakumar and Borst 2006, McCarthy et al. 2007, Hathaway et al. accepted), contributing to microbial pollution in surface waters.

Bioretention has numerous treatment mechanisms for indicator bacteria. In addition to filtering bacteria as stormwater passes through the system, microbes may sorb to organic particles and soils. Such mechanisms result in sequestration of microbes; however, die-off of captured microbes is controlled by other factors. Exposure to sunlight (UV radiation), desiccation, predation, temperature, and nutrient availability can all influence microbial survival (Ferguson et al. 2003, Arnone and Walling 2007). Further, Indicator bacteria have been shown to persist in natural systems. Studies by Sherer et al. (1992) and Jeng et al. (2005) suggest indicator bacteria can persist in sediments from 7 to 30 days given suitable environmental conditions. Therefore, despite treatment mechanisms within bioretention areas to facilitate indicator bacteria removal, microbial persistence within bioretention areas may limit overall effectiveness.

Laboratory analyses emulating bioretention function have been utilized to evaluate the potential for indicator bacteria removal in these systems. Rusciano and Obropta (2007) observed a 91.6% mean reduction of fecal coliform concentrations through bioretention columns receiving diluted swine

manure. Similarly, column studies using conventional bioretention fill media by Zhang et al. (2008) showed an 80% *E. coli* reduction. Zhang et al. (2008) also analyzed bacteria concentrations in the bioretention fill media, observing a 99.9% die off of *E. coli* cells one week after synthetic stormwater runoff was applied to the columns.

Field studies on bioretention have also evaluated indicator bacteria removal. Some studies showed either concentration reductions of indicator bacteria greater than 85% (Hathaway et al. 2009, Passeport et al. 2009) or effluent indicator bacteria concentrations below detectable limits (Dietz and Clausen 2005). Conversely, an analysis of two bioretention cells in Maryland by Li and Davis (2009) yielded somewhat different results. *E. coli* concentration reductions in the two cells were 57% and 0%, while fecal coliform reductions were 0% and 50%. Li and Davis (2009) also observed export of indicator bacteria during some monitored events. Thus, although studies such as Hathaway et al. (2009) have proposed the effectiveness of bioretention for indicator bacteria sequestration, variability exists among field collected performance data. It should be noted that other than Hathaway et al. (2009), field studies performed on bioretention for indicator bacteria have involved seven or fewer samples.

Although there are a growing number of studies evaluating bioretention performance for indicator bacteria, relatively little is understood regarding microbial dynamics within bioretention fill media (Li and Davis 2009). No studies have been performed to evaluate which environmental conditions within bioretention areas can influence indicator bacteria performance. Such data will result in a refined understanding of differences in performance observed for infiltration-based SCMs, and may lead to revised design standards for bioretention being implemented in watersheds with microbial TMDLs.

The objectives of this study were to build upon the current understanding of indicator bacteria removal in bioretention by: (1) evaluating the performance of bioretention areas with varied depths of fill soil, and (2) characterizing physical and chemical properties which may potentially lead to differences in performance between the two bioretention cells.

6.3 Materials and Methods

6.3.1 Site Descriptions

The experimental site was located in Wilmington, North Carolina (Figure 6.1). Two bioretention areas were constructed adjacent to one another within a parking lot (Figure 6.2). A paired-watershed experimental design was desired, but watershed areas differed due to microtopography within the parking lot. The surface areas of the bioretention cells differed by only 1 m^2. One bioretention was constructed with an average soil depth of approximately 60 cm (Bioretention-D), the other had an average soil depth of approximately 25 cm (Bioretention-S). All fill soil for the bioretention areas came from on-site sandy soil, which was classified as Baymeade fine sand (NRCS 2010). Clay and silt comprised 8 to 10 percent of the soil used as fill for the bioretention areas (Table 6.1). This is a lower percentage than bioretention areas evaluated by Dietz and Clausen (2005) and Li and Davis (2009), where fines comprised 16 to 46 percent of the soil media. However, this percentage of fines is acceptable per North Carolina SCM design regulations (NCDENR 2007).

Each cell was constructed with a 10-cm underdrain to facilitate sample collection. It should be noted that underdrains are typically not required for bioretention areas in the sandy soils of coastal areas, thus this design differs from standard practice in the region. Runoff entered each bioretention cell as sheet flow. A small flume was installed at the pavement edge in a location presumed to be representative of the entire watershed. This allowed some pooling of runoff as it entered the bioretention cell, facilitating sampling of the inlet. A similar sampling strategy was used in such studies as Hunt et al. (2006). The bioretention areas were vegetated with turf grass and a small number of shrubs. It should be noted that bypass of the shallow cell occurred on some occasions due to watershed topography routing water around the cell. This bypass was judged to not substantially influence the results of this study. General characteristics of each SCM are given in Table 6.1.

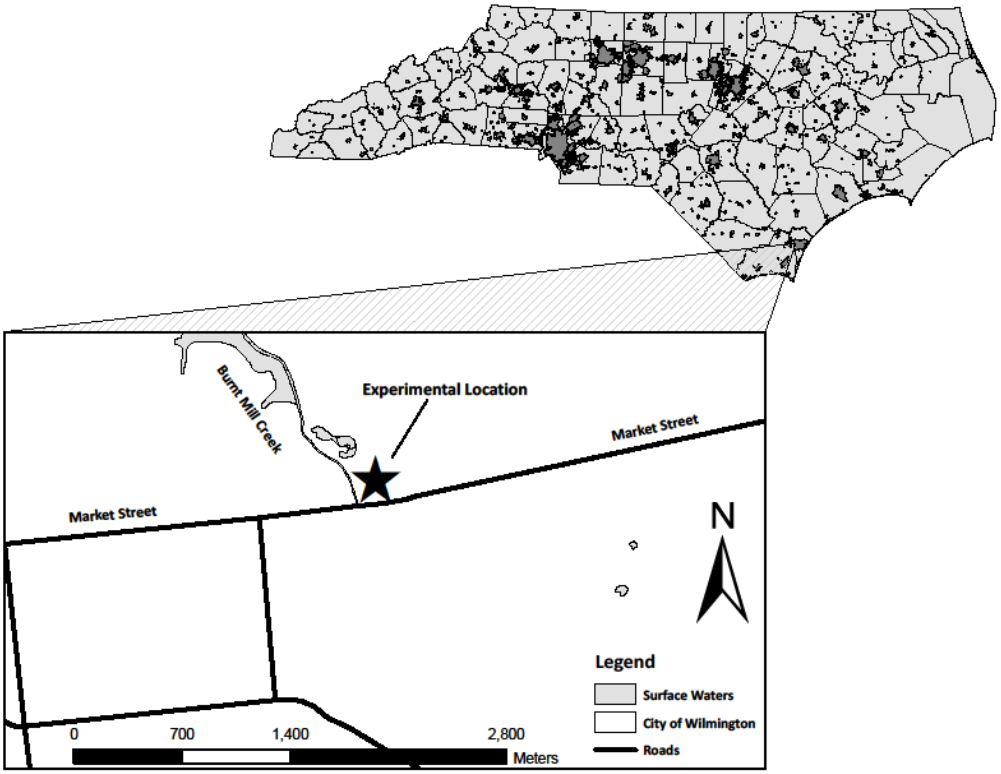

Figure 6.1: Experimental location in Wilmington, NC

Table 6.1: General characteristics of Wilmington SCMs

Characteristic		Bioretention-D	Bioretention-S
Drainage Area (ha)		0.10	0.05
Watershed Composition		Commercial (parking lot)	Commercial (parking lot)
Estimated Imperviousness		100%	98%
Surface Area (m^2)		55	54
Surface Area: Drainage Area Ratio		0.054	0.110
Storage Depth (cm)		28 (1% slope on cell)	28 (1% slope on cell)
Estimated Average Soil Depth (cm)		25	60
Soil Texture[3]	Sand (%)	88	87
	Silt (%)	5	4
	Clay (%)	5	4

1. NRCS 2010 – Soil Data Mart
2. Average depth represents soil depth for bioretention cells
3. Does not add to 100% due to some gravel particles

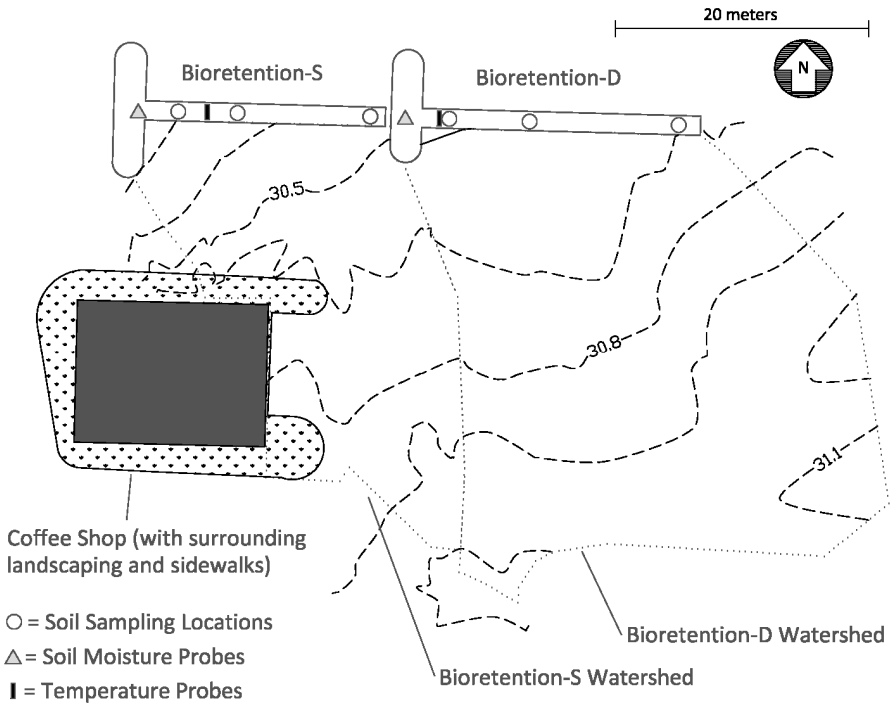

Figure 6.2: Schematic of SCMs and associated watersheds (elevations based on relative datum)

6.3.2 Monitoring Methods – Flow and Rainfall Monitoring

Bioretention underdrains were hydrologically separate and each routed to a nearby catch basin. Two weir boxes fitted with 30-degree, v-notch weirs were installed in the catch basin to receive discharge from each underdrain (independently). Flow was monitored by measuring stage within each weir box using ISCO 6712 autosamplers equipped with 730 bubbler modules. Data were recorded on 5 minute intervals from January 2007 to December 2008, after which data were recorded on 2 minute intervals. An ISCO 674 tipping bucket rain gage was installed at the site, away from the tree canopy, to monitor rainfall. An additional Davis tipping bucket rain gage equipped with a HOBO event logger was installed nearby (approximately 60 m) to provide back-up rainfall data. The ISCO rain gage was not operational until July 2007, leading to the use of data from the Davis rain gage from January 2007 to July 2007. After July 2007, data from the ISCO rain gage was primarily used for rainfall characterization.

Inflow was estimated using the initial abstraction methodology, similar to that employed for estimating inflow to bioretention areas by Li et al. (2009). The Bioretention-D watershed was 100% impervious, while the Bioretention-S watershed was approximately 98% impervious. An initial abstraction of 1 mm (CN of 98) was applied to all impervious areas, and an initial abstraction of 24 mm (CN of 68) was applied to all pervious areas. The amount of rainfall directly falling onto each cell was also calculated and included in the inflow volume.

6.3.3 Monitoring Methods – Indicator Bacteria Monitoring

Grab samples were taken to characterize bioretention performance for indicator bacteria. This is a common methodology for sampling surface waters for indicator bacteria (USEPA 2002, Burton and Pitt 2002). There are valid concerns over the use of grab samples due to potential variations in microbial concentrations during the course of a stormwater runoff event. However, short hold times, increased man-hours, and the technical difficulty of using automatic samplers for microbial analyses led to the use of grab samples for this study. Further, studies such as McCarthy et al. (2008) have illustrated the uncertainties present in indicator bacteria field monitoring, which potentially overshadow the negative impacts of using single grab samples to some degree. Inlet samples were collected for both bioretention areas from the inlet flume discussed previously. Outlet samples were collected from each respective bioretention cell's weir box effluent. Each sample set consisted of two sterile bottles for two bacterial analyses (*E. coli* and enterococci).

Samples were transported to Tritest, Inc for analysis. Hold times were generally less than 6 hours. Samples were analyzed for both *E. coli* and enterococci. *E. coli* were enumerated using Colilert® and enterococci were enumerated using Enterolert®. Each methodology is based on the use of a defined substrate media (IDEXX Laboratories Inc., Westbrook, Maine). Sample dilutions were performed as needed to adequately characterize bacteria concentrations. The Limit of Detection (LOD) was typically either 2 or 10 MPN / 100 ml depending on the dilution utilized. A Maximum Reporting Limit (MRL) was only reached on one occasion and only for enterococci. The MRL was 4839 MPN / 100 ml. Data are analyzed herein using the values at the reporting limit without adjustment.

6.3.4 Monitoring Methods – Physical Measurements

Physical characteristics which could influence bacteria sequestration and persistence in the two bioretention areas were examined. These characteristics included soil temperature, soil moisture, soil chemical properties, and soil physical properties. Soil temperature was monitored in each bioretention cell using temperature sensors (TMCX-HD, accuracy: ±0.5^{o}C at 20^{o}C) connected to HOBO 4-channel data loggers (H08-008-04). Soil moisture was monitored using soil moisture sensors (S-SMC-M005, accuracy: ±0.031 m^3/m^2) connected to HOBO Micro Station Data Loggers (H21-002). Both temperature and soil moisture sensors were placed at approximately 10 and 20 cm in the shallow cell and at 20 cm and 61 cm in the deep cell. Sensors were placed in relatively similar locations in each cell as shown in Figure 6.2. Temperature and soil moisture data were collected on 5-minute intervals and averaged hourly to facilitate analysis.

On 12/15/2008, soil samples were collected from three locations in each bioretention cell at 2 to 3 depths per location (2 for the shallow cell, 3 for the deep cell). Samples were taken to the North Carolina Division of Agriculture and Consumer Services for analysis. Samples were measured for cation exchange capacity (CEC), pH, and other chemical properties. Soil sampling locations are shown in Figure 6.2.

Worm burrows could serve as conduits to facilitate runoff travel through soil media, thereby reducing contact time. A methodology to determine worm burrow abundance in agricultural fields was employed by Fox et al. (2008), and a similar approach was applied at the SCMs in Wilmington, NC, in October 2008. A large fan was attached to the underdrain outlet of both bioretention cells, blowing air upstream into the bioretention soil (Figure 6.3). A smoke generating firework (known as a “smoke bomb”) was released between the fan and the underdrain, causing smoke to be pushed into the cell’s fill media. The smoke bomb lasted approximately 90 seconds. As smoke passed upward through conduits connecting the underdrain to the surface, visible smoke streams emerging from the soil surface were flagged and counted.

Figure 6.3: Fan attached to bioretention underdrain to blow smoke into the bottom of a bioretention cell

6.3.5 Monitoring Methods – Soil Bacteria Analysis

Soil samples were collected for bacteria analyses once per season. A 10 cm auger equipped with clean plastic sampling tubes was used to take soil cores at incremental depths through the soil profile at three locations per bioretention cell (Figure 6.2 – Approximately the same as chemical analysis sampling locations). Approximately 2.5 – 5 cm of turf and soil were removed from the bioretention surface prior to sampling to ensure proper auger function. Equipment was washed with sterile water at each new sampling location. Cores (intact in plastic sleeves) were placed in plastic bags and stored on ice during transport to the Department of Soil Science at North Carolina State University.

Twenty mg of soil from both the top and bottom of each soil column were removed and analyzed separately. Soil samples were suspended in Winogradsky salt solution (10 ml/g soil) (Pochon 1954) and shaken for 15 minutes at 250 rpm on a G10 Gyrotory Shaker (New Brunswick Scientific Company Inc, Edison NJ) at room temperature. The soil suspension was centrifuged (model RC5C, Sorvall Instruments, DuPont) at 2,500 rpm for 10 minutes at 4°C. After centrifugation, 100 ml of supernatant from each sediment sample was analyzed for most probable number (MPN) concentrations of *E. coli* and enterococci by use of the Colilert and Enterolert defined substrate

method, respectively, using the Quantitray/2000 format (Idexx Corporation, Westbrook, ME). All analyses were performed per manufacturer's instructions. Analyses included suitable blanks and standard positive cultures (*E. coli,* ATCC 25922 and *Enterococcus faecium* ATCC 35667) for quality control purposes. Results were reported as MPN of *E. coli* or enterococci per gram of sediment.

6.3.6 Statistical Evaluations

Multiple analyses were utilized to establish the efficiency of indicator bacteria sequestration in the bioretention cells including: (1) removal percentages, (2) effluent concentration comparisons, and (3) probability plots. Removal percentages (Concentration Reduction "CR") were calculated for each SCM using Equation 1. Geometric mean effluent indicator bacteria concentrations from each cell were compared to values from literature and USEPA target concentrations for indicator bacteria in fresh waters. Last, concentration data were used to generate effluent probability plots using methodologies by Burton and Pitt (2002).

$$CR = \left(1 - \frac{\text{Geometric\_Mean}_{outlet}}{\text{Geometric\_Mean}_{inlet}}\right) \times 100\% \qquad (1)$$

All statistical analyses were performed using SAS 9.1 (SAS 2001). Non-parametric Wilcoxon Signed Rank tests were used to determine differences among paired observations. Non-parametric analyses also lessen the influence of high and low concentrations, which is important when data sets contain values below the MDL or above the MRL.

6.4 Results and Discussion

6.4.1 Bioretention Performance for Indicator Bacteria

Twenty storm events were monitored for indicator bacteria between February 2008 and February 2010. Geometric mean influent *E. coli* and enterococci concentrations were 130 MPN / 100 ml and 375 MPN / 100 ml, respectively. The inlet *E. coli* concentration was higher than median values reported at the inlets of two bioretention areas studied by Li and Davis (2009), and less than the geometric mean inlet *E. coli* concentration for a bioretention area evaluated in Hathaway et al.

(2009). The inlet enterococci concentration was similar to that reported by Jones et al. (2008) for a parking lot in New Hampshire. To validate the inlet sampling location, composite grab samples were also collected from multiple inflow points on 18 occasions for *E. coli* and 17 occasions for enterococci. There was no significant difference between the composite samples and samples collected from the inlet flume ($p < 0.05$). Summary statistics are presented in Table 6.2.

Bioretention-S geometric mean effluent concentrations were higher than those from Bioretention-D for both *E. coli* and enterococci, leading to dissimilar concentration reductions. Concentration reductions of *E. coli* and enterococci for Bioretention-D were 70% and 89%, respectively. Concentration "reductions" of *E. coli* and enterococci for Bioretention-S were -119% and -102%, respectively. A statistically significant difference between inlet and outlet concentrations was only found for enterococci in Bioretention-D ($p < 0.05$). No significant difference in inlet and outlet *E. coli* concentrations was found for either SCM ($p < 0.05$). However, significant differences were also found between Bioretention-D and Bioretention-S effluent concentrations for both *E. coli* and enterococci ($p = 0.0158$ and $p = 0.0005$, respectively).

Comparisons to USEPA target indicator bacteria concentrations in fresh waters were performed. The USEPA target concentration for *E. coli* in fresh water is 126 / 100 ml, and the target concentration for enterococci in fresh water is 33 / 100 ml (USEPA 1986). The Bioretention-D geometric mean effluent concentration was lower than the USEPA target concentration for *E. coli* and slightly higher than the target concentration for enterococci. Bioretention-S had a geometric mean effluent concentration higher than the USEPA allowable concentration for both *E. coli* and enterococci. Thirteen of 20 *E. coli* samples and 10 of 20 enterococci samples were lower than USEPA target values at the Bioretention-D outlet. Conversely, at the outlet of Bioretention-S, only 7 of 20 *E. coli* samples and 3 of 20 enterococci samples were lower than USEPA target values. Wilcoxon Signed Rank analyses showed no statistical difference between USEPA target concentrations and effluent indicator bacteria concentrations for Bioretention-D and statistically significant differences for both indicator bacteria for Bioretention-S ($p < 0.05$).

Table 6.2: Comparison of Wilmington, NC, bioretention cells to other field-analyzed sites

Cell Name	Statistic	# of Samples	fecal coliform (per 100 ml)		*E. coli* (per 100 ml)		enterococci (per 100 ml)		Reference
			inlet	outlet	inlet	outlet	inlet	outlet	
Bioretention - D	Geometric Mean	20	-	-	130	39	375	39	-
Bioretention - S	Geometric Mean	20	-	-	130	284	375	378	-
CP	Median	8	140	290	92	90	-	-	Li and Davis (2009)
SS	Median	5	8	2	5	58	-	-	Li and Davis (2009)
Bioretention	Geometric Mean	19 (14 for *E. coli*)	2420	258	241	20	-	-	Hathaway et al. (2009)
Rain Garden 1	n/a	6	< 10	< 10	-	-	-	-	Dietz and Clausen (2005)
Rain Garden 2	n/a	6	< 10	< 10	-	-	-	-	Dietz and Clausen (2005)
North Cell	Mean	7	4172	125	-	-	-	-	Passeport et al. (2009)
South Cell	Mean	4	4172	646	-	-	-	-	Passeport et al. (2009)
Bioretention Area	Median	9	-	-	-	-	400	20	Jones et al. (2008)

Effluent concentrations from Bioretention-D and Bioretention-S were also compared to those reported in other field studies in Table 6.2. Relatively few field studies have been performed for indicator bacteria removal in bioretention areas, particularly for *E. coli* and enterococci. However, Bioretention-D compared well to effluent concentrations reported in literature for both *E. coli* and enterococci. Conversely, Bioretention-S had substantially higher concentrations for both *E. coli* and enterococci.

Effluent probability plots were used for detailed comparison of indicator bacteria concentrations from all monitoring points (Figures 6.4 and 6.5). From these probability plots, differences in performance between Bioretention-D and Bioretention-S are observed, particularly in comparison to USEPA target indicator bacteria concentrations for fresh waters. Estimations of non-exceedance probability were made based on these probability plots. For Bioretention-D, non-exceedance

probabilities for *E. coli* and enterococci were 63% and 47%, respectively. For Bioretention-S, non-exceedance probabilities for *E. coli* and enterococci were 42% and 10%, respectively.

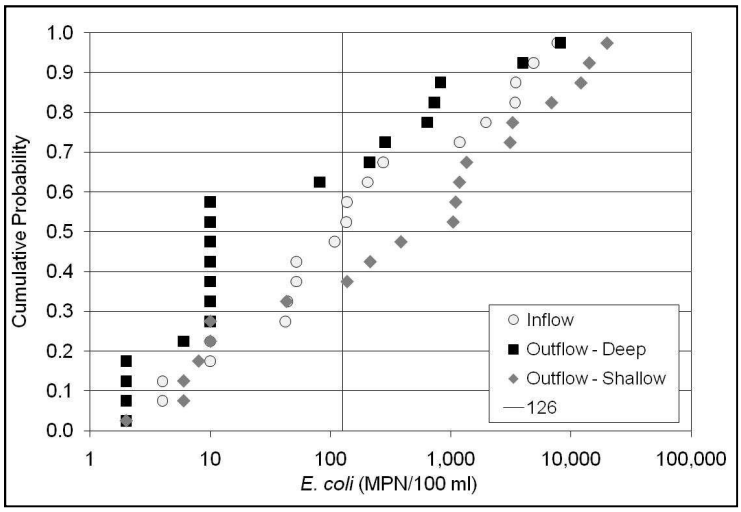

Figure 6.4: *E. coli* cumulative probability plot

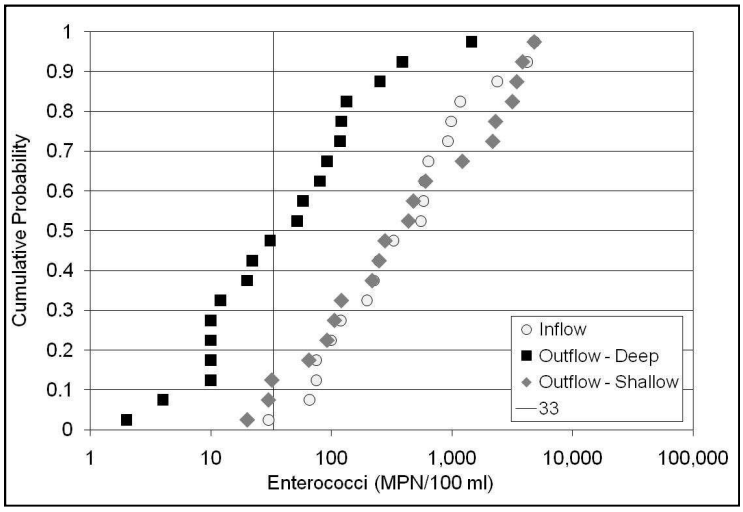

Figure 6.5: Enterococci cumulative probability plot

Based on concentration reductions, comparison to values reported in literature, and comparisons to USEPA standards, Bioretention-D clearly performed well relative to Bioretention-S. Differences in performance could be due to numerous factors which influence microbial survival, including temperature, soil moisture, soil chemistry, and hydrology (Ferguson 2003, Garbrecht et al. 2009).

Thus, efforts were made to characterize various aspects of each bioretention area to determine potential explanations for the difference in performance between the two cells.

6.4.2 Hydrology

Hydrology within the two bioretention cells was characterized over 110 storm events from February 2007 to December 2009. Storm events ranged from 0.03 to 8.7 cm. Outflow typically did not occur for storms less than 1 mm. Qualitative observation of the effluent hydrographs from each of cells indicated differences in functionality between the two systems. Bioretention-S commonly exhibited a sharp spike in effluent flow soon after outflow began, followed by a rapid decline after peak flow was reached. Bioretention-D also exhibited relatively sharp spikes in outflow soon after outflow began; however, Bioretention-D typically had a longer time to peak and outflow continued after Bioretention-S flow had ceased. Obviously, variations in hydrographs were noted based on rainfall patterns. Such variations in outflow hydrograph are logical given the difference in soil depth in the two systems. The deeper Bioretention-D would provide better detention of runoff, allowing slow release over a longer period of time relative to Bioretention-S.

Similar volume reductions were noted for the two bioretention cells after summing total estimated inflow and outflow over the storms monitored. Bioretention-D and Bioretention-S provided infiltration of approximately 63% and 61% of monitored runoff, respectively. Outflow volume was normalized by watershed area for direct comparison of the two systems. Figure 6.6 shows the cumulative probability plot for effluent volume (normalized by area) for the two cells. Functionality of the two systems was similar throughout much of the probability plot, and a Wilcoxon Signed Rank analysis indicated no significant difference among normalized outflow from the two SCMs ($p < 0.05$). Some variation existed during smaller flow events, where the deep system produced outflow more often. This may be due to measurement errors at low flows or a proportionally higher volume delivery from the larger watershed during small events.

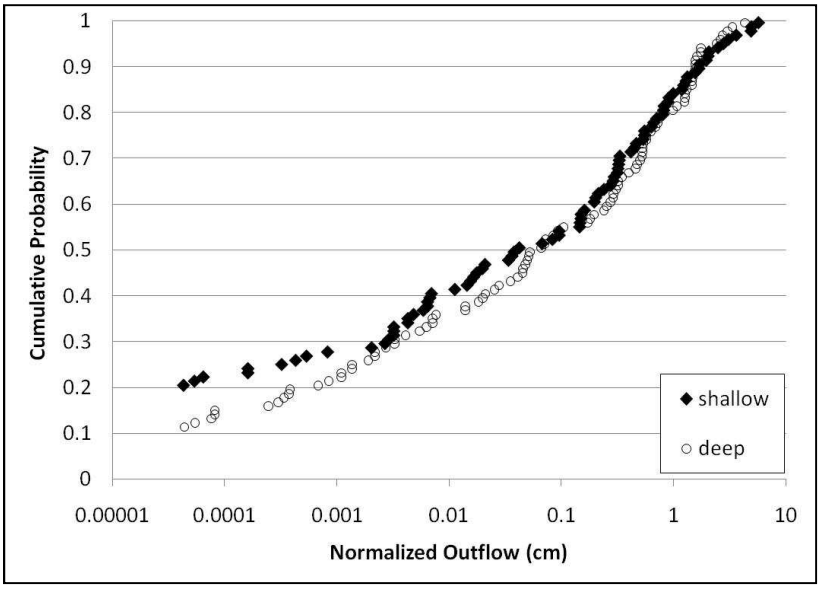

Figure 6.6: Effluent volume cumulative probability plot

Effluent peak flows from each cell were also normalized by watershed area to account for hydrologic differences in the influent runoff. The cumulative probability plot for normalized peak flow from the two bioretention cells is presented in Figure 6.7. Hydrologic differences between the two cells are more apparent based on Figure 6.7. Normalized peak flow from Bioretention-S was higher than that from Bioretention-D for a substantial proportion of storm events. This was supported by Wilcoxon Signed Rank analyses where statistical differences were found between normalized peak flow from the two cells ($p < 0.05$). Similar observations of increased peak flow mitigation with increased soil media depth in bioretention were made by Li et al. (2009).

These analyses are consistent with qualitative observations of the effluent hydrographs, where sharp peaks were noted for Bioretention-S. Thus, Bioretention-S seemed to support little detention of stormwater runoff. Studies such as Li and Davis (2009) suggest bioretention hydrology can influence pollutant removal by affecting such factors as contact time. Further, assuming similar infiltration capacities of the soils and ponding depths in the two cells, the substantially shallower Bioretention-S likely supports a higher flux and consequently, higher interstitial velocity. This may be important in explaining differences in performance between the two cells as retention of indicator

bacteria has been shown to decrease as flow velocities increase in soil columns (Garbrecht et al. 2009).

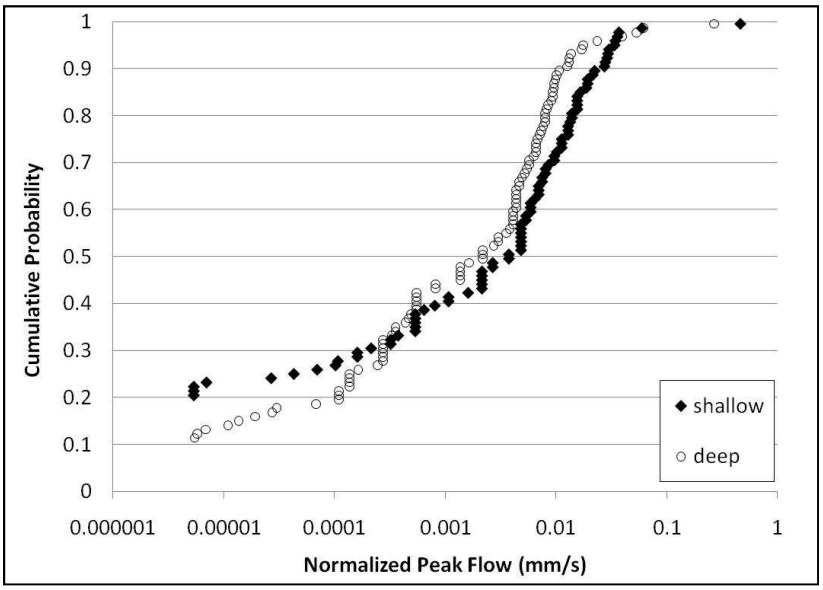

Figure 6.7: Effluent peak flow cumulative probability plot

6.4.3 Worm Hole Presence

While a study of worm presence was not conducted, potential worm burrows were quantified by filling the underdrains with smoke and identifying locations where smoke was leaving the soil surface in concentrated streams. Very few worm holes, or other conduits, were identified. Eight smoke releases were identified in Bioretention-S and 0 were found in Bioretention-D. This indicates Bioretention-S had slightly more direct connection from the surface to the underdrain through which water could short circuit, thus negating much of the filtering benefit offered by the soil media. However, the abundance of holes was not particularly high, and the results suggest only a modest potential for flow-through in the more shallow Bioretention-S.

6.4.4 Soil Temperature and Moisture

Temperature data were collected between January 2009 and October 2009. Equipment malfunctions left missing data for some portions of the monitoring period. Characterization of

temperature and soil moisture probes was performed by averaging data collected at probes from two depths in each bioretention cell. Average monthly temperatures for Bioretention-D and Bioretention-S are presented in Table 6.3. As expected, the deeper Bioretention-D showed a lower, more buffered average temperature throughout the majority of the study. Average temperatures in Bioretention-S reached as high as 34.4^{o}C and as low as 7.0^{o}C, while Bioretention-D reached as high as 31.3^{o}C and as low as 7.6^{o}C. Similarly, Jones and Hunt (2009) suggest deeper bioretention areas are more conducive to thermal control due to the temperature stability in deeper soils. Jones and Hunt (2009) also showed maximum soil temperatures above 30^{o}C in monitored bioretention areas.

Table 6.3: Average monthly soil temperatures in Bioretention-S and Bioretention-D

Month	Bioretention-S Average Temperature (^{o}C)	Bioretention-D Average Temperature (^{o}C)
January	11.1	10.7
February	12.3	11.2
March	15.6	14.0
April	21.3	19.3
Mid-July[1]	28.5	26.3
August	31.0	28.7
Beginning-September[2]	28.8	27.2
End-October[3]	22.4	20.7

1: 7/7/2009 – 7/13/2009
2: 9/1/2009 – 9/4/2009
3: 10/24/2009 – 10/29/2009

It should be noted that some variability was observed between temperature probes placed at a depth of approximately 20 cm in each cell. This may be due to the presence of an underdrain immediately under a depth of 20 cm in Bioretention-S, potentially allowing warming of the soils due to ambient air entering the underdrain. Temperature variations may have also occurred due to measurement accuracy of the probes themselves. Nonetheless, the average temperature in the Bioretention-S cell over the entire monitoring period was found to be only 1.5 ^{o}C higher than Bioretention-D. The difference in hourly average temperature between the two SCMs was statistically significant ($p < 0.05$).

Soil moisture data were collected from January 2009 to January 2010. Equipment malfunctions left missing data during some portions of the monitoring period. Figure 6.8 shows the hourly average soil water content in both bioretention cells. The decline in soil moisture following storm events followed a similar pattern in both bioretention cells. Bioretention-S typically had higher soil moisture content throughout the study. Despite a smaller watershed area, Bioretention-S had a higher average runoff loading when inflow was normalized by soil volume. Thus, more runoff was available to saturate soils within Bioretention-S. Average Bioretention-S water content was approximately 0.04 m^3/m^3 higher than that of Bioretention-D. The difference in average hourly soil moisture between the two SCMs was statistically significant ($p < 0.05$).

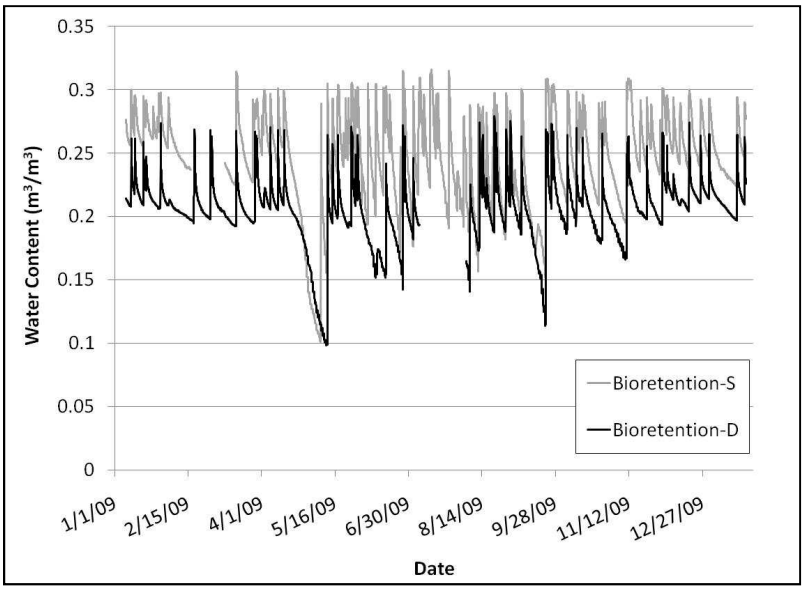

Figure 6.8: Average hourly soil moisture in Bioretention-S and Bioretention-D

A laboratory evaluation of *E. coli* in soils by Chandler and Craven (1980) identified moisture availability as a crucial factor affecting survival. Byappanahalli et al. (2006) analyzed beach sand amended with plankton in laboratory studies, showing significant increases in *E. coli* over the first 24 hours of the study followed by a gradual return over the next 6 days to levels approximately 1-log over initial concentrations. Byappanahalli et al. (2006) theorized that indicator bacteria in moist soils have the potential to grow during warm summer months when nutrients are available. Similar assertions were made by Byappanahalli and Fujioka (1998) regarding *E. coli* in Hawaiian soils, where

adequate temperature, moisture, and nutrients conditions appeared to allow growth. Thus, the warmer and wetter soil environment present in Bioretention-S (relative to Bioretention-D) may provide a more favorable environment for indicator bacteria growth. However, influences on microbial growth are complex and involve numerous factors. In a review of transport and fate processes involving microbes, Ferguson et al. (2003) concluded that few studies have examined more than one or two factors simultaneously. Further, confounding relationships in literature are present in regard to microbial persistence vs. microbial growth. Kibbey et al. (1978) showed S. faecalis survival (persistence) was prolonged as soil temperature *decreased* and soil moisture increased. Thus, it is unknown to what degree relatively small variations in soil temperature and water content influence indicator bacteria concentrations in bioretention areas. Additionally, soil moisture conditions likely vary spatially within bioretention areas depending on underdrain location, bioretention slope (if present), and inlet location. Overall, differences in temperature and soil moisture in the two systems appeared relatively minor, but slightly favored microbial growth and/or persistence in Bioretention-S.

6.4.5 Soil Properties

Although soils were characterized for numerous soil properties (Table 6.4), not all are directly related to microbial performance. Microbial sequestration is influenced by soil properties such as pH and soil type (Ferguson et al. 2003). Soil pH varied from 6.4 to 8.0 in Bioretention-S and from 7.6 to 8.0 in Bioretention-D, with average pH being 7.5 and 7.9 in Bioretention-S and Bioretention-D, respectively. Thus, differences in soil pH appeared relatively minor. Coyne (1999) describes a pH range of 6 to 7 as optimal for *E. coli*, with a pH of 9 as a maximum. Similar data were reported for soils from two bioretention areas studied by Li and Davis (2009), where pH for the two cells was reported as 7.3 and 7.7. Overall, soils were similar between the two cells with regard to phosphorus index, cation exchange capacity, humic matter, and pH, all of which are common parameters used to describe soils.

As previously noted, bioretention cells studied in Wilmington, NC, had high sand content and relatively low fines (clay and silt) relative to studies by Li and Davis (2009) and Dietz and Clausen (2005). This likely influenced the performance of the cells in regard to indicator bacteria sequestration. Column studies by Mankin et al. (2007) utilized sand and silt loam soils to test *E. coli*

sorption, finding greater sorption for silt loam soils. Studies by Garbrect et al. (2009) also evaluated differences in soil type with regard to *E. coli* sequestration. Garbrect et al. (2009) demonstrated enhanced performance by loamy sands (84.5% sand) in comparison to course sands (100% sand). Soils in Bioretention-S and Bioretention-D were very similar in regard to distribution between sand, silt, and clay. Thus, differences in performance based on soil type do not seem to be logical.

Table 6.4: Soil properties of Bioretention-S and Bioretention-D

Location		Approximate Depth Range Collected From (cm)	Cation Exchange Capacity (meq / 100 cm^3)	Soil pH	Phosphorus Index	Humic Matter (%)
Bioretention-S	Site 1	0 - 7.6	11.4	6.4	34	0.3
		20.3 - 30.5	11.2	7.4	41	0.3
	Site 2	0 - 7.6	8.4	7.6	23	0.3
		14.0 - 24.1	4.1	7.8	13	0.1
	Site 3	0 - 7.6	14.8	7.8	24	0.2
		10.2 - 20.3	8.7	8	12	0.2
Average =			**9.8**	**7.5**	**24.5**	**0.2**
Bioretention-D	Site 1	0 - 7.6	7.2	8	20	0.3
		17.8 - 22.9	6	8	17	0.3
		48.3 - 53.3	32.6	8.2	9	0.1
	Site 2	0 - 7.6	12.9	7.7	41	0.5
		17.8 - 22.9	3.2	7.6	21	0.4
		58.4 - 63.5	7.1	8	19	0.2
	Site 3	0 - 7.6	13.2	7.8	56	0.5
		22.9 - 30.5	11.1	7.9	49	0.4
		55.9 - 61.0	9.2	7.9	31	0.4
Average =			**11.4**	**7.9**	**29.2**	**0.3**

6.4.6 Soil Indicator Bacteria

Multiple collection tubes were used to sample the soil profile in each bioretention cell. Indicator bacteria were measured at the top and bottom of each tube; however, data presented in Tables 6.5 and 6 include only the top and bottom of the first tube and the bottom of each tube thereafter. Approximate depths are presented for each reading, but actual depths varied based on sampling date.

Data were spatially and temporally variable for both *E. coli* and enterococci, making trends difficult to identify. However, some observations can be made. First, enterococci soil bacteria concentrations were consistently and markedly higher than *E. coli* concentrations. Consistent relationships were even more difficult to find in enterococci data. Enterococci are known to be more persistent in the environment (USEPA 2001), potentially leading to higher concentrations. It should also be noted that the geometric mean inlet enterococci concentration was higher than that of *E. coli*. Additionally, geometric mean Bioretention-S effluent enterococci concentrations were higher than those of *E. coli*. Studies such as Hartel et al. (2005) showed the ability of enterococci to regrow after desiccation and rewetting, which would commonly occur in bioretention systems.

Table 6.5: Soil enterococci concentrations in Wilmington, NC, bioretention areas

SCM	Location	Approximate Depth (cm)	enterococci (MPN / g)			
			12/15/2008[1]	3/5/2009[2]	6/1/2009[3]	8/4/2009[4]
Bioretention-S	Site 1	5 - 7.5	> 242.0	101.1	> 242.0	> 242.0
		20	> 242.0	101.1	> 242.0	14.6
		30	> 242.0	96.1	> 242.0	130.0
	Site 2	5 - 7.5	> 242.0	31.3	> 242.0	11.0
		23	92.1	25.1	43.5	3.7
	Site 3	5 - 7.5	43.5	101.1	1.7	4.5
		23	32.6	0.3	8.7	17.3
Bioretention-D	Site 1	5 - 7.5	> 242.0	101.1	> 242.0	6.5
		24	> 242.0	24.0	11.6	21.4
		44	242.0	91.4	3.4	242.0
		53	> 242.0	33.0	3.4	7.4
	Site 2	5 - 7.5	> 242.0	101.1	4.4	25.0
		25	> 242.0	68.9	5.3	64.9
		47	62.9	57.5	16.8	77.0
		61	96.1	96.1	4.2	46.1
	Site 3	5 - 7.5	> 242.0	101.1	> 242.0	> 242.0
		22	198.6	101.1	> 242.0	> 242.0
		39	> 242.0	96.1	196.6	34.5
		51	> 242.0	101.1	-	-

1: Antecedent dry period: 3.5 days, Average temperature preceding 7 days: 11.8^{o}C
2: Antecedent dry period: 4 days, Average temperature preceding 7 days: 6.3^{o}C
3: Antecedent dry period: 2.5 days, Average temperature preceding 7 days: 23.7^{o}C
4: Antecedent dry period: 0.5 days, Average temperature preceding 7 days: 27.3^{o}C

E. coli concentrations were commonly higher in the upper portion of the soil column. Analysis of indicator bacteria in river bank soils by Desmarais et al. (2002) also suggested a decline in *E. coli* concentration with soil depth, yet a less appreciable decline for enterococci. This is consistent with the general understanding that microbial abundance is highest in the top of the soil profile due, in part, to availability of oxygen and nutrients (Coyne 1999). Despite some patterns which could roughly be identified, there is not sufficient evidence to suggest indictor bacteria concentrations are substantially different in the soil of either bioretention cell.

Table 6.6: Soil *E. coli* concentrations in Wilmington, NC, bioretention areas

SCM	Location	Approximate Depth (cm)	*E. coli* (MPN / g)			
			12/15/2008[1]	3/5/2009	6/1/2009	8/4/2009
Bioretention-S	Site 1	5 - 7.5	> 242.0	0.5	2.8	< 0.1
		20	242.0	0.9	0.3	< 0.1
		30	173.3	1.7	0.2	0.1
	Site 2	5 - 7.5	11.9	0.6	0.6	< 0.1
		23	4.6	0.4	1.9	< 0.1
	Site 3	5 - 7.5	0.2	< 0.1	< 0.1	0.1
		23	< 0.1	< 0.1	1.1	< 0.1
Bioretention-D	Site 1	5 - 7.5	0.1	0.5	8.4	< 0.1
		24	< 0.1	0.2	2.5	< 0.1
		44	< 0.1	< 0.1	0.1	0.1
		53	< 0.1	0.2	1.1	< 0.1
	Site 2	5 - 7.5	0.5	0.5	1.4	0.2
		25	0.4	0.1	1.0	0.5
		47	< 0.1	1.4	2.9	0.1
		61	0.3	2.4	0.6	< 0.1
	Site 3	5 - 7.5	< 0.1	0.1	24.9	< 0.1
		22	< 0.1	1.7	36.5	< 0.1
		39	< 0.1	< 0.1	1.2	< 0.1
		51	< 0.1	< 0.1	-	-

1: Antecedent dry periods and temperatures for each sampling date presented in Table 5

Laboratory analysis of bioretention soil columns by Rusciano and Obropta (2007) indentified fecal bacteria primarily in the top 5.1 cm of soil. However, indicator bacteria were present throughout the soil profiles on some sampling dates at the Wilmington, NC, SCMs. No other field studies have been performed where indicator bacteria concentrations were evaluated within bioretention soils,

making comparisons to other field sites impossible. However, it appears that although variability in concentrations were noted between sampling dates, indicator bacteria can be present in the soils of bioretention areas after storm events. Similarly, persistence has been show in stream and estuarine sediments by Sherer et al. (1992) and Jeng et al. (2005). Thus, bacteria are likely present and available to persist, potentially regrow, and be exported during subsequent events. Regrowth studies performed on indicator bacteria in river bank soils by Desmarais et al. (2002) showed increases in concentrations during the first 24 hours of laboratory experiments. Conversely, studies such as Zhang et al. (2008) showed rapid declines in *E. coli* concentration in bioretention columns studied at the laboratory scale. Thus, understanding microbial ecology within bioretention systems is an area of research need within the stormwater management community (Li and Davis 2009). Microbial survival in soils is dependent on numerous environmental factors including (but not limited to) temperature, soil moisture, and predation (Ferguson 2003, Desmarais et al. 2002). Thus, soil conditions after a rain event likely influence persistence and may also influence the ability to identify relationships in these data. It is apparent that higher resolution field studies are required to investigate microbial relationships in bioretention soil than available in these data. Characterizations cannot be made based on only one or a limited number of sampling dates based on the variability present in these data. Also, there are likely environmental phenomena which occur in field studies that cannot be easily replicated in laboratory analyses.

6.4.7 Synthesis of Data and Design Implications

Bioretention-D and Bioretention-S showed differing levels of indicator bacteria sequestration based on multiple performance analysis metrics. Soil chemistry and other soil properties of the two bioretention cells were similar. Higher average temperature and soil moisture within the shallower Bioretention-S suggests the potential for greater regrowth; however, because differences were relatively minor, the authors minimize the significance of these differences in explaining the contrast in performance between the systems. Further, no substantial differences in indicator bacteria concentrations within the two system's soils could be established, suggesting neither is more prone to microbial persistence and/or regrowth (although more research is needed to study microbial interactions in bioretention).

Thus, the difference in soil depth was identified as the most likely factor affecting performance of the two SCMs. Soil depth can influence multiple facets of bioretention function. In addition to reductions in contact time as suggested by Li and Davis (2009), higher soil water flux prevails in shallow systems (assuming similar hydraulic head). Bioretention-S was found to exhibit poorer mitigation of peak flow despite relatively similar performance between the two systems in regard to volume reduction. This suggests poorer detention of flow relative to Bioretention-D. These physical characteristics have implications for microbial performance, as column studies by Garbrect et al. (2009) showed decreased sequestration of *E. coli* occurred in coarse sand columns with increased flow velocity, and column studies by Bright et al. (accepted) demonstrated that total coliform concentrations leaving sand columns decreased over time as infiltration rates decreased.

The influence of hydrologic function in bioretention cells likely varies based on soil type. For instance, observations on *E. coli* sequestration in soil columns by Garbrect et al. (2009) indicated varied performance based on soil type (coarse sand vs. loamy sand). Similar observations were made on soil columns by Mankin et al. (2007), where silt loam was found to have a greater capacity for *E. coli* retention than sand. Therefore, it should be restated that the SCMs studied in Wilmington, NC, had a lower percentage of soil fines than studies by Li and Davis (2009) and Dietz and Clausen (2005). However, soil fines were found to be 8 to 10%, which is within the State of North Carolina's acceptable range for bioretention media (NCDENR 2007).

Determining appropriate bioretention media depth and composition is an ongoing area of research (Davis et al. 2009). Shallow media depths offer economic benefits, as soil media can be a substantial portion of the cost associated with bioretention construction. Further, design guidance such as Hunt and Lord (2006) suggested that shallow bioretention depths are adequate for pathogen/indicator bacteria sequestration due to the supposition that they are removed at the bioretention surface. This assertion was supported by such studies as Rusciano and Obrotpa (2007), where fecal bacteria were only found in the top 5.1 cm of bioretention soil columns in laboratory analyses. However, the data herein suggest indicator bacteria can be present lower in the soil profile, leaving them available for later export from the system. Nonetheless, as bioretention media depth becomes more shallow, a higher fraction of the soil profile consists of depths generally considered to have the greatest

abundance of microbes (Coyne 1999). This, in turn, leaves less soil available for indicator bacteria capture prior to their exiting the bioretention system.

Economics, hydrologic performance, and water quality function must be balanced when determining appropriate bioretention depth. For indicator bacteria sequestration, these data suggest a minimum bioretention depth does exist. Specifically, the authors suggest bioretention should not be less than 60 cm deep when indicator bacteria are a concern. This depth will provide better detention of runoff and decrease soil water flux. It is possible that inclusion of a higher percentage of fines would lead to better performance for indicator bacteria even in very shallow bioretention; however, hydrologic function may be compromised.

6.5 Conclusions

Two bioretention cells were monitored in Wilmington, NC, in a paired-watershed experimental design. The two cells had two different soil depths: Bioretention-D with a soil depth of 60 cm and Bioretention-S with a soil depth of 25 cm. Concentration "reductions" in Bioretention-D and Bioretention-S were 70% and -119% for *E. coli* and 89% and -102% for enterococci, respectively. Geometric mean effluent *E. coli* and enterococci concentrations from Bioretention-D compared relatively well to USEPA target values and to values reported in literature for bioretention. Conversely, geometric mean effluent *E. coli* and enterococci concentrations from Bioretention-S did not compare well to USEPA target values or those reported in literature. Thus, based on multiple performance evaluation metrics, Bioretention-D clearly outperformed Bioretention-S.

Evaluation of multiple factors which could impact performance in the systems was conducted. Soil type and soil chemistry were found to be quite consistent between the two bioretention cells. Conversely, soil temperature and soil moisture were found to be slightly higher in Bioretention-S, potentially leading to slightly more favorable conditions for regrowth. However, no substantial differences in indicator bacteria abundance could be identified in bioretention soils over 4 sampling events. Thus, the most logical difference between the two SCMs, which could lead to differences in performance, is soil depth. Bioretention-S was found to have poorer detention of runoff, and the shallow system would logically result in higher soil water flux. Higher velocity of runoff passing

through the system should result in less contact time and potential stripping of bacteria from the soil matrix.

Although shallow bioretention cells offer numerous design and economic benefits, they may have functional limitations with respect to indicator bacteria. These data suggest design of bioretention for indicator bacteria removal should include a soil media depth of at least 60 cm. This will result in greater indicator bacteria sequestration and runoff detention.

6.6 Acknowledgements

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6.7 References

Arnone, R.D., and J.P. Walling. “Waterborne pathogens in urban watersheds.” *Journal of Water and Health*, 5(1): 149-162.

Bright, T.M., J.M. Hathaway, W.F. Hunt, F.L. de los Reyes, and M.R. Burchell. (accepted). “Impact of stormwater runoff on clogging and fecal bacteria reduction in sand columns.” *Journal of Environmental Engineering*.

Byappanahalli, M.N., R.L. Whitman, D.A. Shively, W.T. Evert Ting, C.C. Tseng, and M.B. Nevers. (2006). “Seasonal persistence and population characteristics of *Escherichia coli* and enterococci in deep backshore sand of two freshwater beaches.” *Journal of Water and Health*, 4: 313-319.

Byappanahalli, M.N. and R.S. Fujioka. (1998). “Evidence that tropical soil environment can support the growth of *Escherichia coli*.” *Water Science and Technology*, 38(12): 171-174.

Chandler, D.S. and J.A. Craven. (1980). "Relationship of soil moisture to survival of *Escherichia coli* and *Salmonella typhimurium* in soils." *Australian Journal of Agricultural Research*, 31: 547 – 555.

Coyne, M.S. (1999). "Introduction to Soil Microbiology." *Delmar Cengage Learning*, Albany, NY.

Davis, A.P., M. Shokouhian, H. Sharma, and C. Minami. (2006). "Water quality improvement through bioretention media: nitrogen and phosphorus removal." *Water Environment Research*, 78(3): 284-293.

Davis, A.P., W.F. Hunt, R.G. Traver, and M. Clar. (2009). "Bioretention technology: overview of current practice and future needs." *Journal of Environmental Engineering*, 135(3): 109 – 117.

Desmarais, T.R., H.M. Solo-Gabriele, and C.J. Palmer. (2002). "Influence of soil on fecal indicator organisms in a tidally influenced subtropical environment." *Applied and Environmental Microbiology*, 68(3): 1165-1172.

Dietz, M.E. and J.C. Clausen. (2005). "A field evaluation of rain garden flow and pollutant treatment." *Water, Air, and Soil Pollution*, 167(1):123-138.

Ferguson, C., A.M. de Roda Husman, N. Altavilla, D. Deere, and N. Ashbolt. (2003). "Fate and transport of surface water pathogens in watersheds." *Critical Reviews in Environmental Science and Technology*, 33(3), 299 – 361.

Fox, G.A., R. Kanwar, and R. Malone. (2008). "Earthworms and *E. coli*." *Resource: Engineering and Technology for a Sustainable World*, 15(6): 22-24.

Garbrecht, K., G.A. Fox, J.A. Guzman, and D. Alexander. (2009). "*E. coli* transport through soil columns: implications for bioretention cell removal efficiency." *Transactions of the ASABE*, 52(2): 481-486.

Hartel, P.G., K. Rodgers, J.A. Fisher, J.L. McDonald, L.C. Gentit, E. Otero, Y. Rivera-Torres, T.L. Bryant, and S.H. Jones. (2005). "Survival and regrowth of fecal enterococci in desiccated and rewetted sediments." *Proceedings of the 2005 Georgia Water Resources Conference*, The University of Georgia, Athens, GA.

Hathaway, J.M., W. F. Hunt, and S. Jadlocki. (2009). "Indicator bacteria removal in storm-water best management practices in Charlotte, North Carolina." *Journal of Environmental Engineering*, 135(12): 1275 – 1285.

Hathaway, J.M., W.F. Hunt, and O.D. Simmons III. (accepted). "Statistical evaluation of factors affecting indicator bacteria in urban stormwater runoff." *Journal of Environmental Engineering*.

Hunt, W.F., A.R. Jarrett, J.T. Smith, and L.J. Sharkey. (2006). "Evaluating bioretention hydrology and nutrient removal at three field sites in North Carolina." *Journal of Irrigation and Drainage Engineering*, 132(6): 600 – 608.

Hunt, W.F. and W.G. Lord. (2006). “Bioretention performance, design, construction, and maintenance.” *North Carolina Cooperative Extension*, Raleigh, NC.

Jeng, H.C., A.J. England, and H.B. Bradford. (2005). “Indicator organisms associated with stormwater suspended particles and estuarine sediment.” *Journal of Environmental Science and Health*, 40: 779-791.

Jones, M.P. and W.F. Hunt. (2009). “Bioretention impact on runoff temperature in trout sensitive waters.” *Journal of Environmental Engineering*, 135(8): 577-585.

Jones, S.H., R. Roseen, R. A. Wildey, J. Maimes, J. Houle, T. Ballestero. (2008). “Enterococci populations in conventional, manufactured, and LID stormwater treatment systems.” *Proc., World Environmental and Water Resources Congress*, ASCE, Reston, VA.

Kibbey, H.J., C. Hagedorn, and E.L. McCoy. (1978). “Use of fecal streptococci as indicators of pollution in soil.” *Applied and Environmental Microbiology*, 35(4): 711-717.

Li, H., and A.P. Davis. (2009). “Water quality improvement through reductions of pollutant loads using bioretention.” *Journal of Environmental Engineering*, 135(8): 567-576.

Li, H., L.J. Sharkey, W.F. Hunt, A.P. Davis. (2009). “Mitigation of impervious surface hydrology using bioretention in North Carolina and Maryland.” *Journal of Hydrologic Engineering*, 14(4): 407-415.

Mankin, K.R., L. Wang, S.L. Hutchinson G.L. Marchin. (2007). “*Escherichia coli* sorption to sand and silt load soil.” *Transactions of the ASABE*, 50(4): 1159-1165.

McCarthy, D.T., V.G. Mitchell, A. Deletic, and C. Diaper. (2007). “*Escherichia coli* in urban stormwater: explaining their variability.” *Water Science and Technology*, 56(11), 27-34.

North Carolina Department of Environment and Natural Resources (NCDENR). (2007). “Stormwater Best Management Practices Manual.” Raleigh, NC.

Passeport, E., W.F. Hunt, D.E. Line, R.A. Smith, and R.A. Brown. (2009). “Field study of the ability of two grassed bioretention cells to reduce storm-water runoff pollution.” *Journal of Irrigation and Drainage Engineering*, 135(4): 505 – 510.

Pochon, J. (1954) “Manuel Technique d'Analyse Microbiologique du Sol” Masson et Cie, Paris, France.

Roseen, R.M., T.P. Ballestero, J.J. Houle, P. Avellenda, R. Widley, and J. Briggs. (2006). “Storm water low-impact development, conventional structure, and manufactured treatment strategies for parking lot runoff: performance evaluations under varied mass loading calculations.” *Transportation Research Record: Journal of the Transportation Research Board*, No. 1984, 135-147.

Rusciano, G.M. and C.C. Obropta. (2007). “Bioretention column study: fecal coliform and total suspended solids reductions.” *Transactions of the ASABE*, 50(4), 1261-1269.

Selvakumar, A., and M. Borst. (2006). “Variation of microorganism concentrations in urban stormwater runoff with land use and seasons.” *Journal of Water and Health*, 4(1): 109-124.

Sherer, B.M., J.R. Miner, J.A. Moore, J.C. Buckhouse. (1992). “Indicator bacteria survival in stream sediments.” *Journal of Environmental Quality*, 21: 591-595.

State Climate Office of North Carolina (SCONC). (2010). “NC CRONOS Database (KILM)” http://www.nc-climate.ncsu.edu/cronos/?station=KILM&temporal=D (June 7, 2010).

United States Environmental Protection Agency (USEPA). (1986). “Ambient water quality criteria for bacteria – 1986.” *EPA 440/5-84-002*. Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA). (2000). “Low impact development (LID): a literature review.” *EPA-841-B-00-005*, Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA). (2001). “Protocol for developing pathogen TMDLs.” *EPA-841-R-00-002*. Office of Water, Washington, DC.

United States Environmental Protection Agency (USEPA). (2010). “Water quality assessment and total maximum daily loads information (ATTAINS). “ http://www.epa.gov/waters/ir/index.html (May 5, 2010).

Zhang, L, E.A. Seagren, and A.P. Davis. (2008). “Column studies on the capture and destruction of *E. coli* from simulated urban stormwater runoff using conventional bioretention media and iron oxide-coated sand.” *Proc., World Environmental and Water Resources Congress*, ASCE, Reston, VA.

7 Summary and Future Research

Microbial quality in surface waters is a concern across the United States, Europe, Australia, and elsewhere due to human reliance on surface waters for food, recreation, and other life sustaining activities. Although pathogens are of utmost concern, indicator bacteria are typically used for regulatory purposes to indicate the presence of fecal matter, and thus the possible existence of pathogens. Total Maximum Daily Loads (TMDLs) are established for surface waters impacted by excessive indicator bacteria. Analyses are required to categorize sources of indicator bacteria, and a plan is developed to restore water quality in the impacted water by way of various management/control practices. Stormwater runoff has been shown to have high indicator bacteria concentrations, contributing to microbial degradation in surface waters.

Although numerous studies have been performed to establish patterns of indicator bacteria transport and export in estuarine and riverine systems, relatively little research has been performed for urban stormwater (prior to runoff entering surface water). An analysis was performed to determine which variables influence indicator bacteria export from an urban watershed in Raleigh, NC. Event Mean Concentrations (EMCs) of *E. coli* and fecal coliform exhibited significant seasonal variation based on Kruskal-Wallis analyses ($p < 0.05$). Based on multiple linear regression analyses, EMCs were also influenced by antecedent meteorological conditions, with temperature and moisture being important in explaining variability among sampling events. This study emphasized the importance of seasonality and antecedent conditions in indicator bacteria transport and export from urban watersheds. Seasonal differences must be accounted for in TMDLs. These data suggest that seasonal variations should be carefully considered when estimating indicator bacteria export from urban areas due to the large differences in EMCs observed between seasons.

Further analysis provided a traditional first flush assessment of data collected from the urban watershed. Although total suspended solids (TSS) exhibited a first flush in the watershed, no first flush effect was noted for *E. coli* and enterococci, and the first flush effect for fecal coliform was relatively weak. Seasonal variations in first flush strength were observed, likely due to differences in

pollutant sources between seasons. Stormwater Control Measures ("SCMs" - also known as Best Management practices or "BMPs") are designed under the assumption that a first flush effect exists. However, the lack of a substantial first flush effect suggests SCMs cannot sequester proportionally more indicator bacteria as a result of greater mass delivery during the beginning of storm events.

Watershed analyses were only performed on one watershed in Raleigh, NC. Thus, variations in trends due to watershed characteristics are likely. For example, regional differences in climate may result in differences in correlations involving meteorological factors. First flush analyses were also potentially influenced by indicator bacteria persistence in stormwater pipes. Thus, first flush characteristics may vary in small, impervious watersheds where stormwater pipes are not present. Similar studies performed in watersheds in other locations and with differing characteristics would allow a greater understanding of the reproducibility of these results.

Stormwater runoff is typically managed by implementation of SCMs. Although SCMs have been shown to sequester numerous pollutants, relatively little is known regarding their ability to sequester indicator bacteria. The effectiveness of SCMs in Charlotte, NC, and Wilmington, NC, was examined. Differences in performance were noted between the two locations, potentially due to differences in particle association of indicator bacteria between the relatively clayey soils in Charlotte, NC, and the sandy soils in Wilmington, NC. High water tables in Wilmington, NC, likely also influenced results, particularly for wet ponds, where dilution of stormwater runoff due to groundwater intrusion was likely. Although some SCMs showed statistically significant reductions of indicator bacteria ($p < 0.05$), some SCMs appeared to export indicator bacteria. These data suggest SCMs do possess treatment mechanisms which are effective at sequestering indicator bacteria; however, an environment may be present in some SCMs which allows indicator bacteria to persist and/or regrow. In general, bioretention provided the most consistent sequestration of indicator bacteria across both locations. Further, infiltration-based SCMs offer some advantage, as mass removal of indicator bacteria can be realized through infiltration of runoff into subsoils.

Further study is needed to determine the impact of infiltrated stormwater on groundwater systems. Although infiltration of stormwater runoff may lead to reduced export to surface waters, little is

known regarding the fate of microbes in groundwater being fed by SCMs. SCMs constructed similarly and employing similar mechanisms of pollutant removal (wetland, wet ponds, etc.) exhibited varied performance for indicator bacteria in both this study and others in literature. More research is needed to determine why such variability is present. For instance, stormwater wetlands were shown to perform well in Charlotte, NC, but not Wilmington, NC. Although inferences can be made as to the cause behind these differences in performance, a refined understanding of microbial processes in these systems would allow a greater understanding of potential design modifications to SCMs which may lead to improved performance. If no such design modifications are possible, SCMs equipped with different or new treatment mechanisms may be required to treat microbes in stormwater runoff.

For the Wilmington, NC, SCMs, effluent indicator bacteria concentrations were observed to vary seasonally, although no significant relationships could be found ($p < 0.05$). Trends were apparent for both *E. coli* and enterococci for all but one SCM. The geometric mean effluent enterococci concentration was slightly higher during the non-swimming season for one wetland. These data suggest the potential for variations in indicator bacteria export from SCMs during warmer seasons. Such seasonal differences, if present, must also be considered in microbial TMDLs. However, due to the lack of statistically significant relationships identified in these data, additional study is needed to verify the trends identified herein. Additional research should also be focused toward determining if these apparent differences in effluent indicator bacteria concentrations are due to elevated influent concentrations or poorer sequestration of indicator bacteria during warmer months.

A paired watershed study in Wilmington, NC, showed differing performance between two bioretention cells constructed with varied media depth. Differences in function were potentially attributable to numerous factors, including differences in soil temperature, soil moisture, soil chemistry, and soil physical properties. These factors were evaluated, with the only notable differences between the cells being differing media depth and a slightly warmer and moister environment in the shallow bioretention area. The differences in temperature and moisture were not considered substantial enough to result in such dramatic differences in performance. Thus, soil media depth was identified as the most likely difference between the two cells leading to

differences in indicator bacteria sequestration. This is due to the influence of media depth on hydrology within the system, with a shallow depth leading to higher soil water flux. This leads to reduced hydraulic contact time, and possibly stripping of bacteria from the soil matrix. For bioretention cells, a minimum soil media depth appears to exist, below which poor sequestration of indicator bacteria may occur due to high soil water flux and low contact time.

Soil water flux appears to be an important consideration during bioretention design. Although an increase in soil media depth is one option for decreasing soil water flux, other design options may provide similar results. A reduction in ponding depth, and subsequent increase in surface area, will also lead to decreased soil water flux. Stormwater may also be slowed in bioretention areas by decreasing infiltration rate. This may be accomplished by increasing the content of fines within bioretention media. Increasing the percentage of fines may also lead to increased sorption of indicator bacteria. Thus, more shallow media depths may be possible provided the soil media utilized has a higher fraction of fine soils (clay and silt). However, such modification to soil media will also result in decreased hydrologic efficiency. Thus, the trade-off between indicator bacteria sequestration and hydrologic efficiency should be carefully considered. These design options should be explored in future research.

APPENDIX

A. Appendix: Watershed Hydrographs with Sampling Events

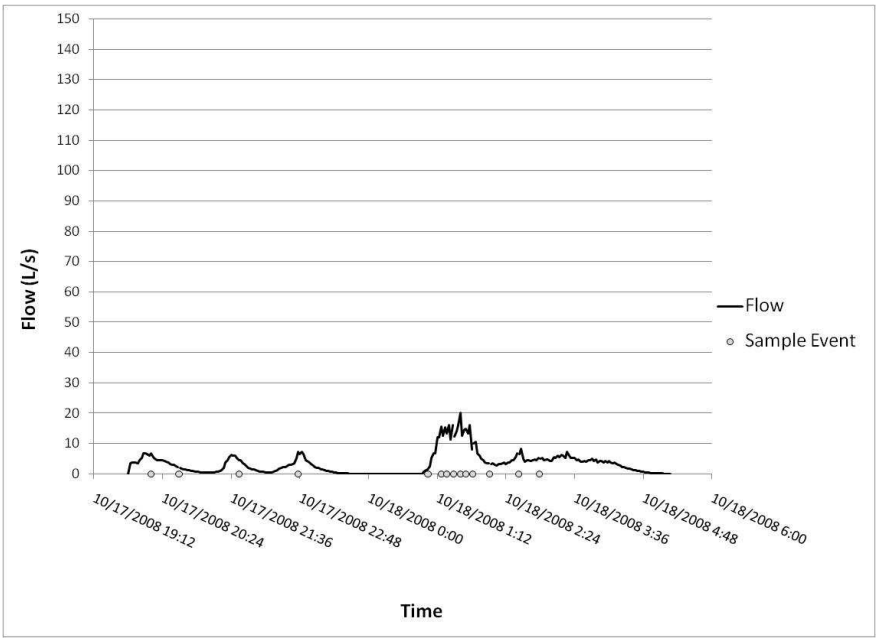

Figure A.1: Flow and sampling events during 10/17/2008 storm

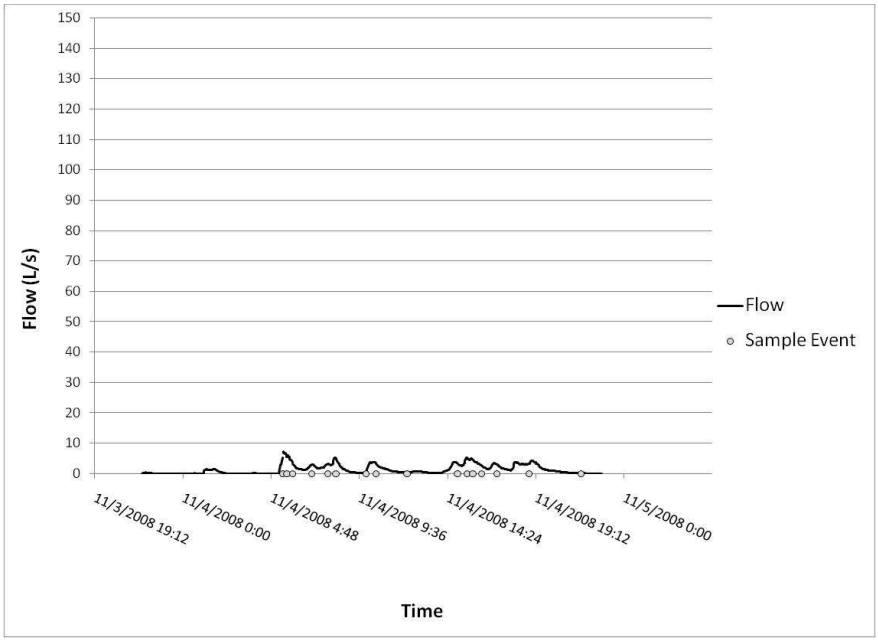

Figure A.2: Flow and sampling events during 11/04/2008 storm

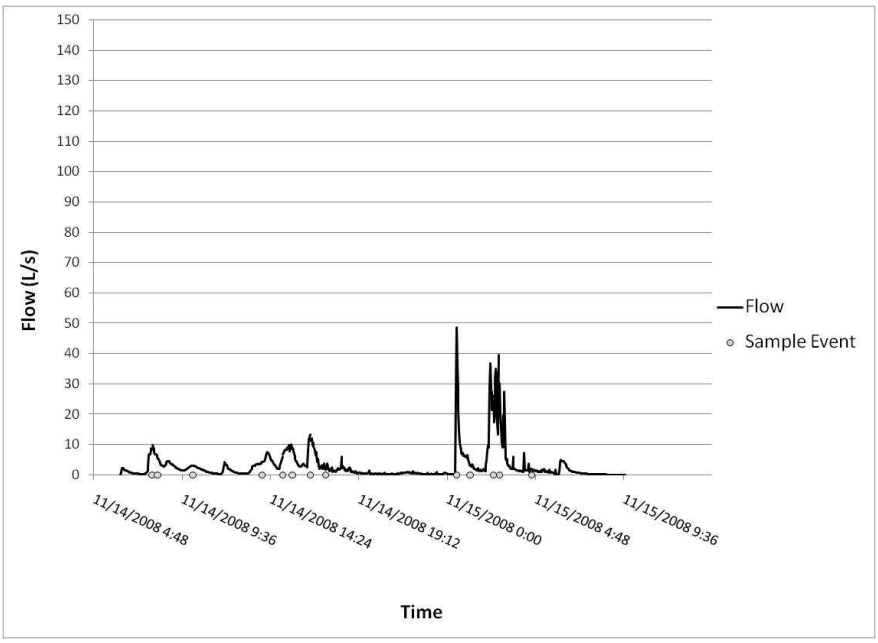

Figure A.3: Flow and sampling events during 11/14/2008 storm

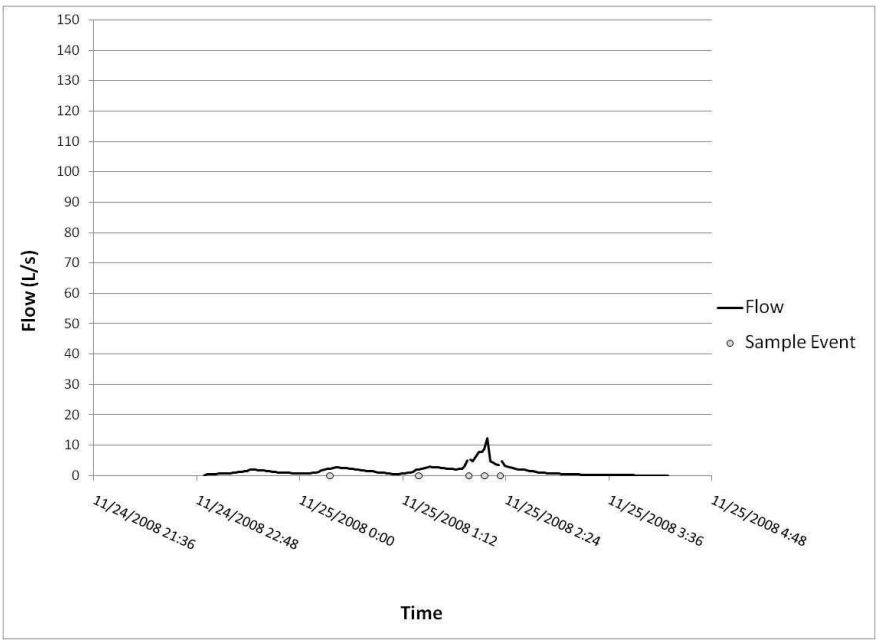

Figure A.4: Flow and sampling events during 11/25/2008 storm

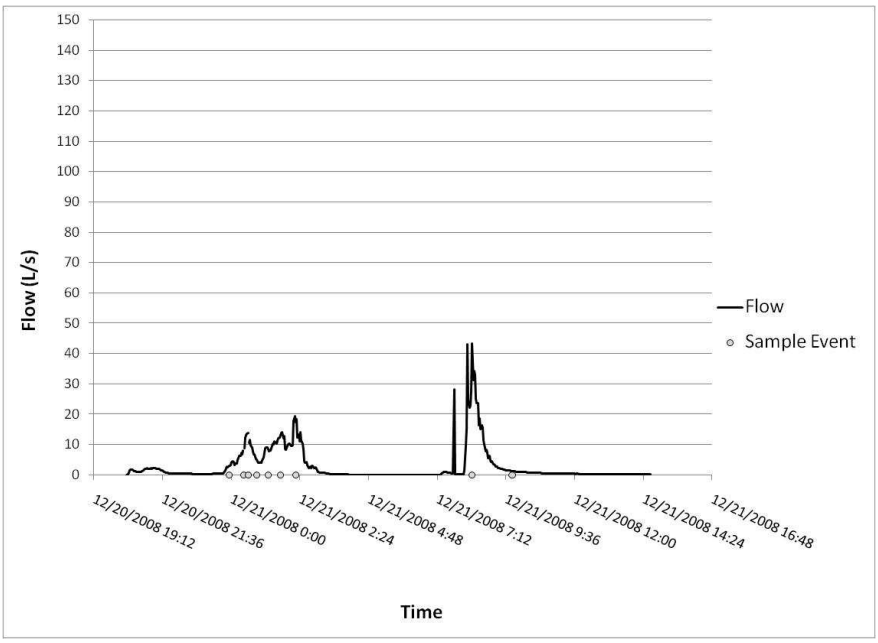

Figure A.5: Flow and sampling events during 12/20/2008 storm

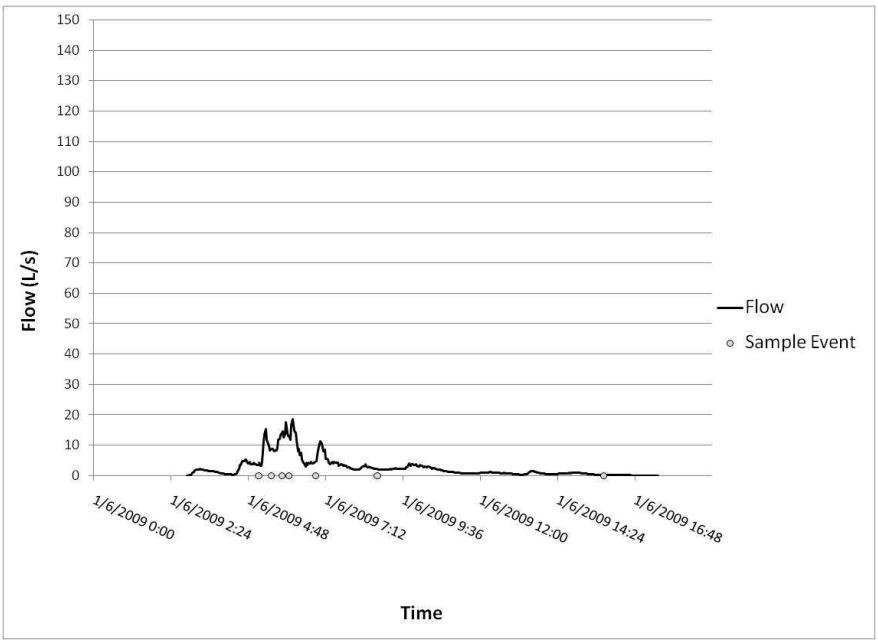

Figure A.6: Flow and sampling events during 1/06/2009 storm

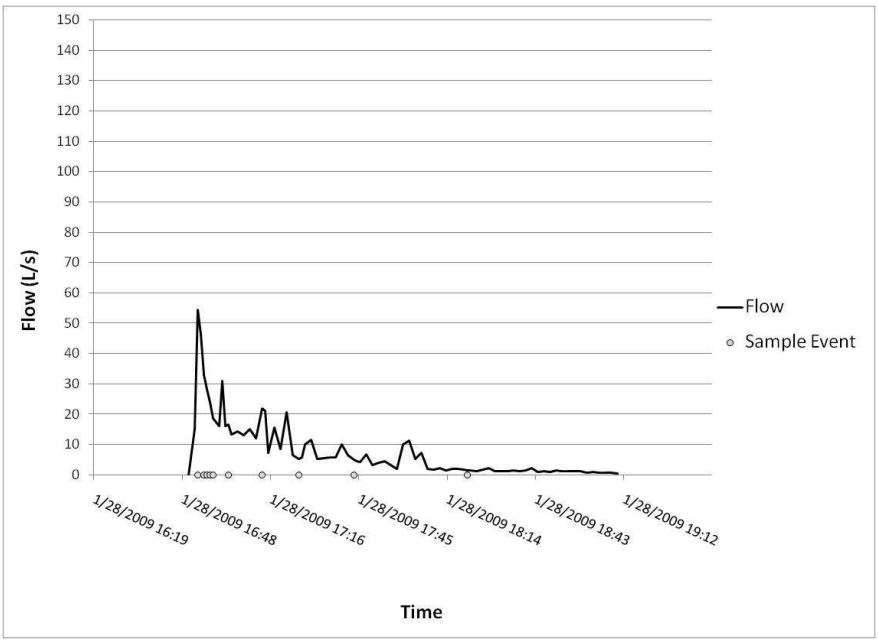

Figure A.7: Flow and sampling events during 1/28/2009 storm

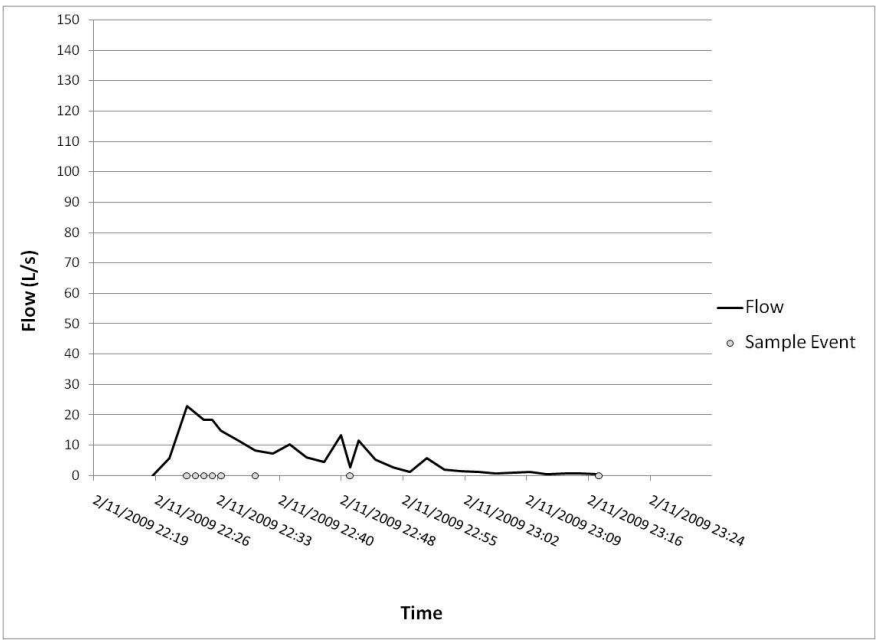

Figure A.8: Flow and sampling events during 2/11/2009 storm

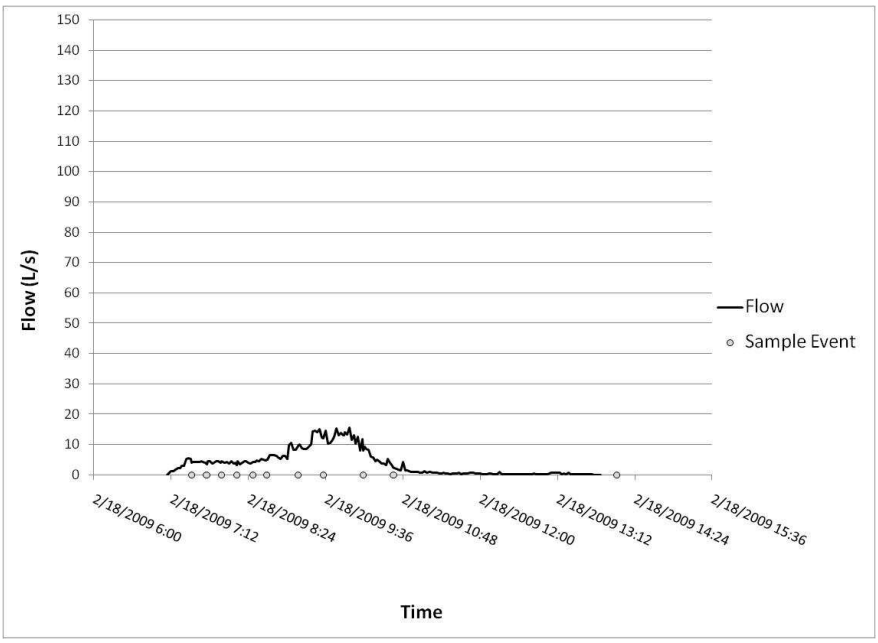

Figure A.9: Flow and sampling events during 2/18/2009 storm

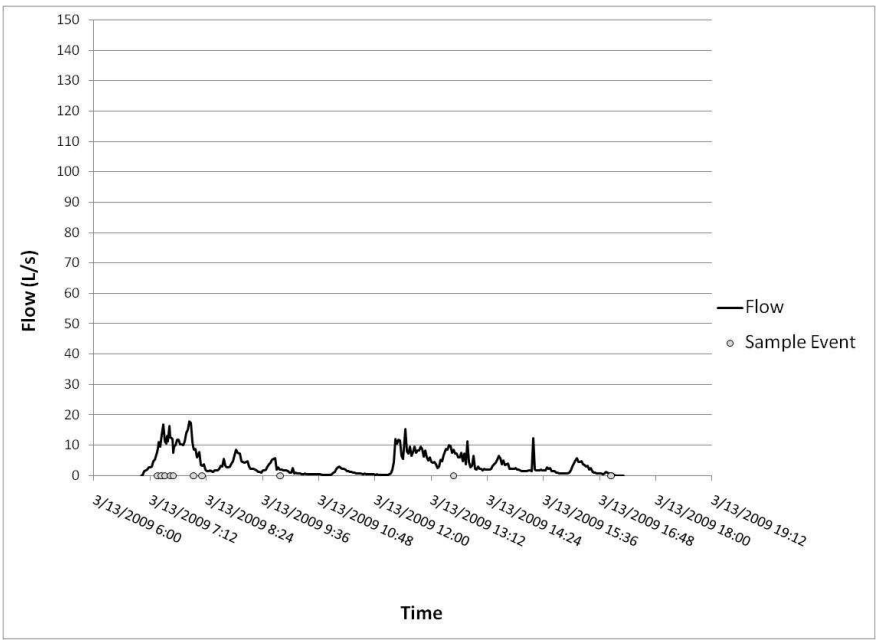

Figure A.10: Flow and sampling events during 3/13/2009 storm

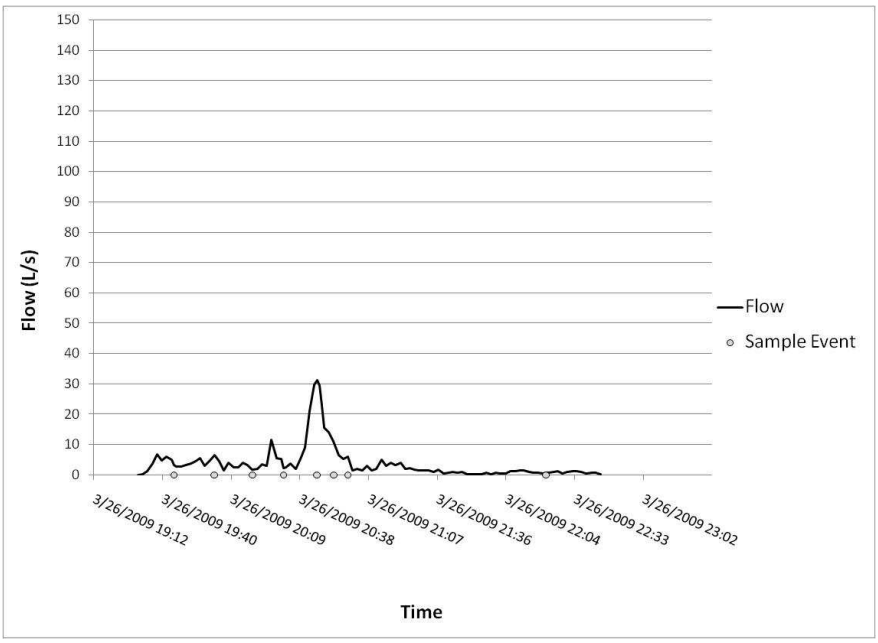

Figure A.11: Flow and sampling events during 3/26/2009 storm

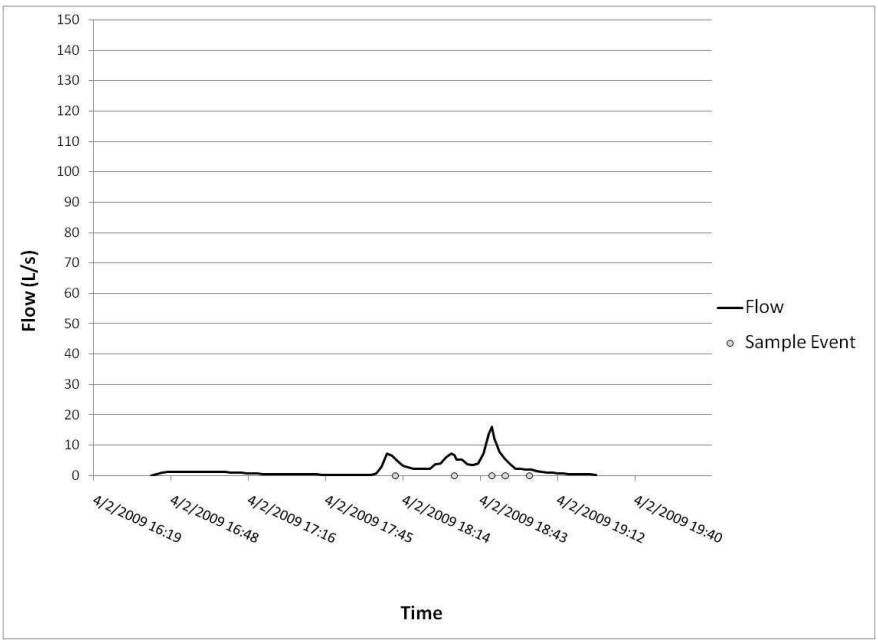

Figure A.12: Flow and sampling events during 4/02/2009 storm

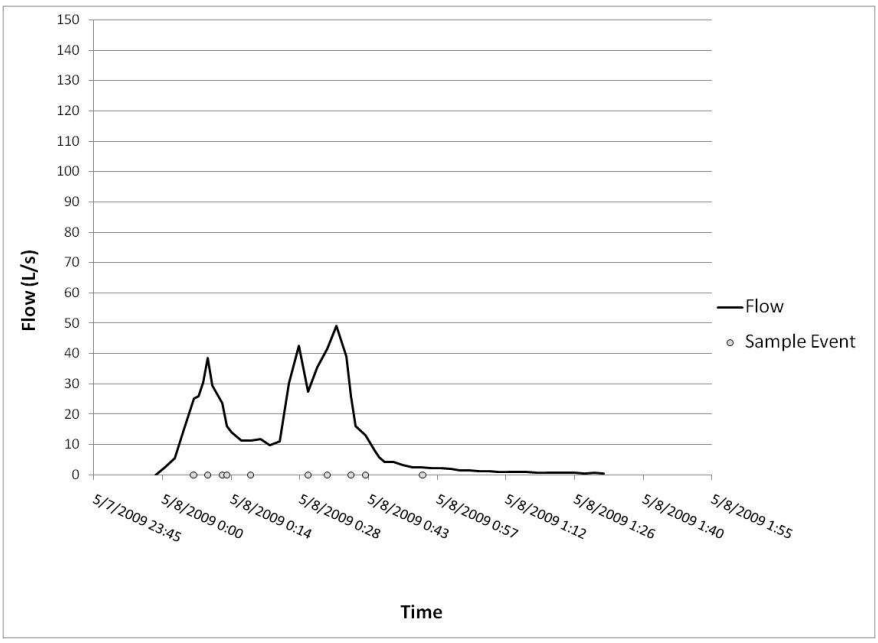

Figure A.13: Flow and sampling events during 5/08/2009 storm

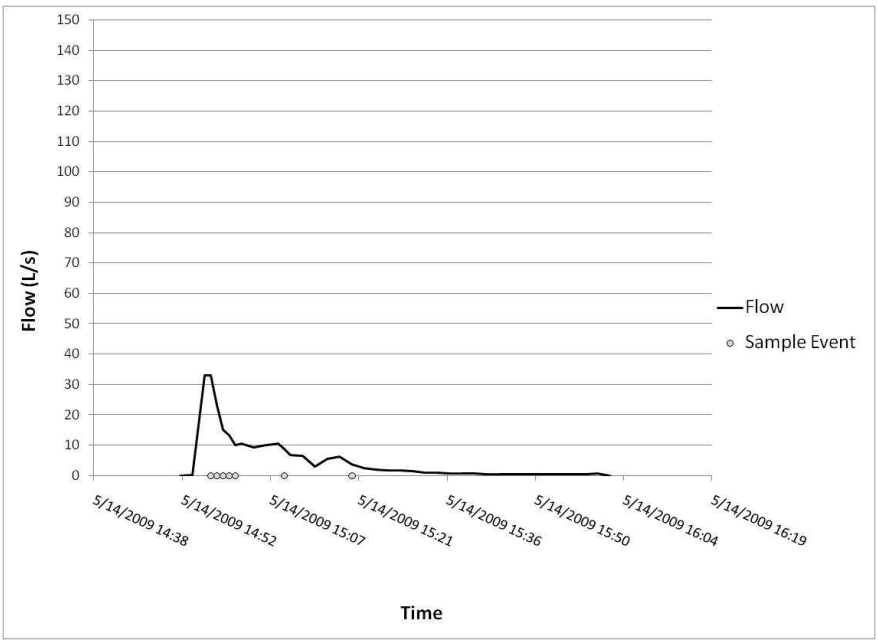

Figure A.14: Flow and sampling events during 5/14/2009 storm

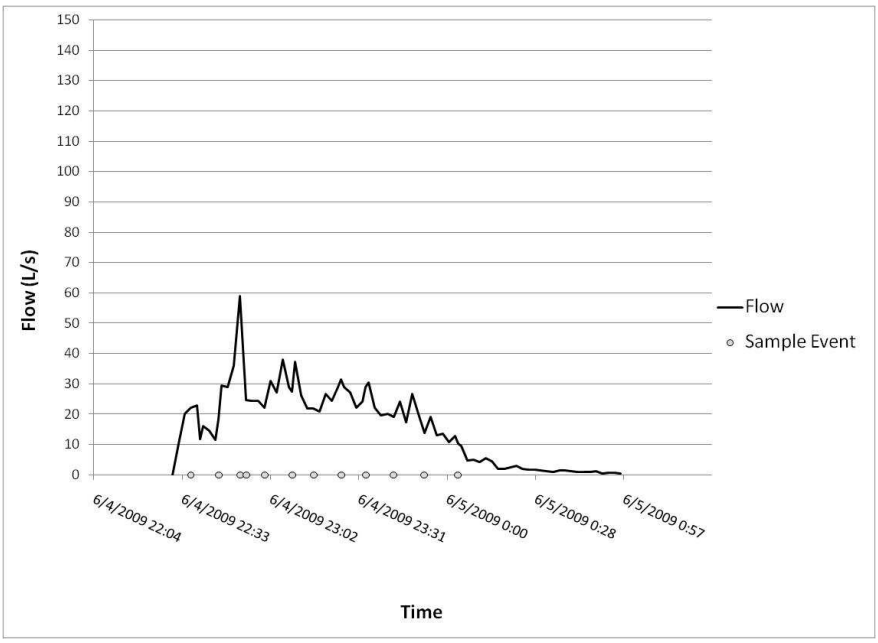

Figure A.15: Flow and sampling events during 6/04/2009 storm

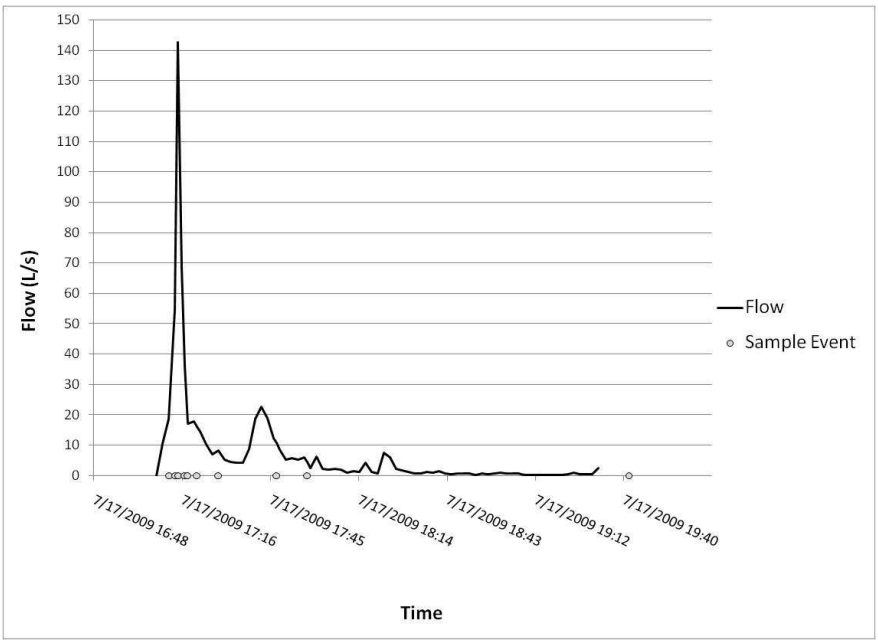

Figure A.16: Flow and sampling events during 7/17/2009 storm

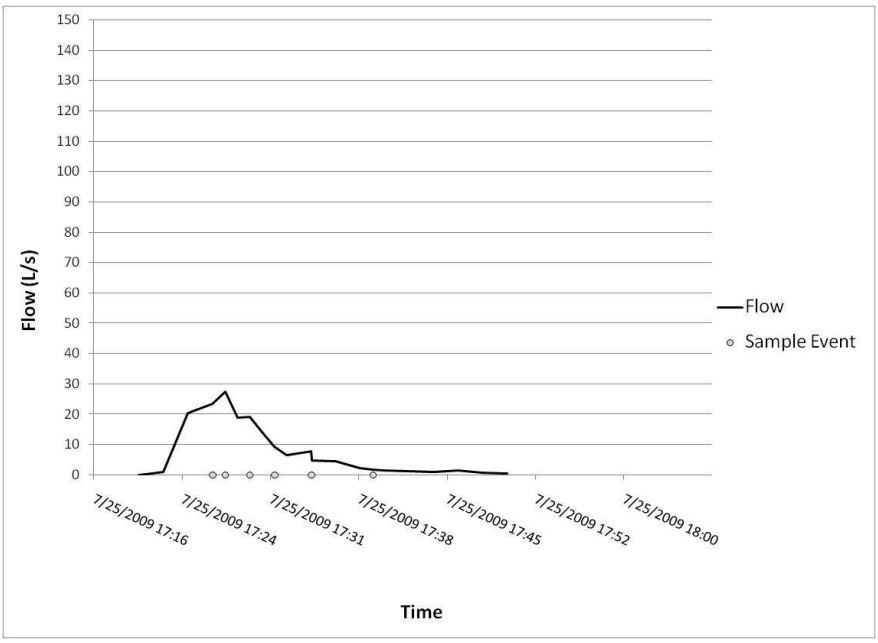

Figure A.17: Flow and sampling events during 7/25/2009 storm

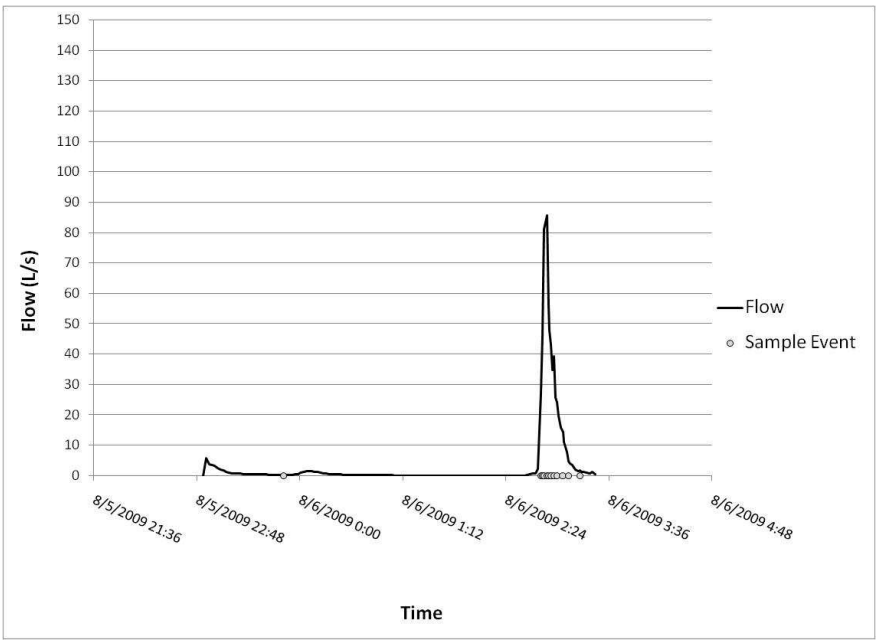

Figure A.18: Flow and sampling events during 8/05/2009 storm

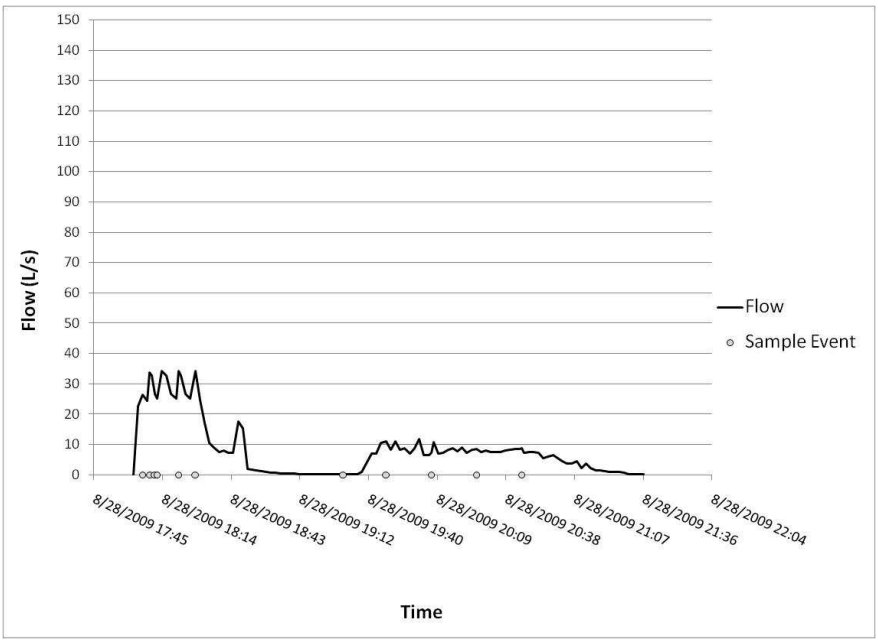

Figure A.19: Flow and sampling events during 8/28/2009 storm

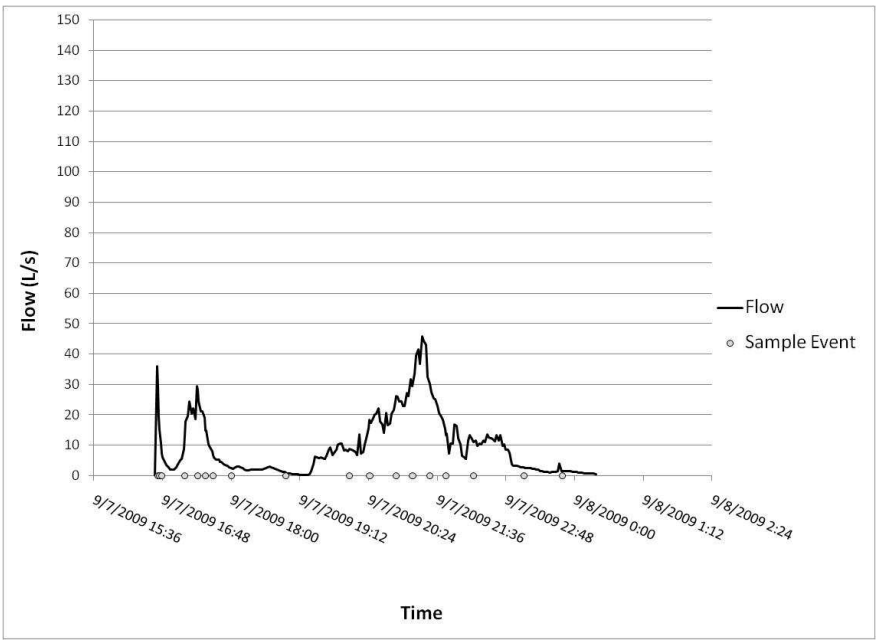

Figure A.20: Flow and sampling events during 9/07/2009 storm

B. Appendix: Bacteria Analysis Results for Raleigh, NC, Watershed

Table B.1: Discrete bacteria concentrations 10/17/2008 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	6873	6362	149170	134600	636
2	6739	5248	90061	89683	750
3	4964	7431	655460	274742	8382
4	8031	12070	55876	70073	13082
5	2167	2038	655460	377906	1722
6	14053	4157	79266	60333	3651
7	20373	26993	103582	87456	3941
8	19773	32774	111877	80671	3139
9	44652	55429	84393	138481	1096
10	55876	73845	133691	132258	1473
11	111877	63009	655460	116565	1358
12	96398	112216	121685	177217	2099
13	47220	58246	103582	132258	520
14	44652	53760	111877	82569	967

Table B.2: Discrete bacteria concentrations 11/04/2008 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	15171	18401	39941	45089	958
2	32095	31592	55876	55429	2991
3	26143	29643	70280	52885	4835
4	21701	34168	37788	45089	2000
5	23762	20212	42228	32774	2894
6	20223	14732	47220	60835	9208
7	19034	20212	44652	83424	3050
8	16557	21674	42228	55508	3417
9	18988	15889	52820	68754	3557
10	8746	12187	28678	55508	2292
11	10044	11063	59124	60835	2572
12	11221	9756	44652	42620	3506
13	18333	9756	39941	37458	2762
14	12087	6305	42228	43551	2762
15	9493	7431	44652	38993	1859
16	15483	21674	59124	77204	6006

Table B.3: Discrete bacteria concentrations 11/14/2008 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	14249	14586	121685	72920	15171
2	13226	6248	103582	50503	14489
3	15171	17585	55876	72287	14977
4	4236	1009	33868	25428	7292
5	6006	7499	37788	42620	8537
6	5207	8581	28903	47505	14249
7	7446	5248	33868	29643	8777
8	12973	19998	62589	61898	15346
9	7208	8661	170985	87775	11269
10	14246	6305	121685	20212	18333
11	13811	10854	55876	74106	11656
12	15483	6305	47565	31219	13564
13	22695	24444	52820	58246	17770

Table B.4: Discrete bacteria concentrations 11/25/2008 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	1840	1009	16557	20432	850
2	4876	3087	20553	23438	7561
3	3699	3087	13226	12306	4803
4	2964	8661	9229	23176	7446
5	4509	7431	12256	17402	6941

Table B.5: Discrete bacteria concentrations 12/20/2008 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	4785	5248	62589	82569	5807
2	6941	6362	28903	32774	7352
3	14489	16051	22695	42620	10032
4	11221	13578	25182	31388	7505
5	6750	9756	18341	20212	9518
6	6190	5248	10620	10958	3995
7	8301	3034	16010	18401	7772
8	14762	12306	24909	21674	12600
9	14977	17585	21701	25007	20553

Table B.6: Discrete bacteria concentrations 1/6/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	6417	8661	11269	11063	7561
2	5644	6248	8382	7364	37788
3	4642	1009	8382	4157	16424
4	5376	12187	7938	16051	14977
5	3362	2038	4388	7499	10670
6	4785	6362	7446	9849	12973
7	2043	100	4785	6362	10036

Table B.7: Discrete bacteria concentrations 1/28/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	19089	25148	21701	32926	10430
2	17721	17050	24909	24172	15647
3	11269	9666	15483	10854	11877
4	7666	8661	8746	11064	9576
5	10992	12070	59125	119203	6739
6	9229	8661	9229	11064	5207
7	7446	7364	10044	9666	5727
8	7208	9849	8159	11064	5904
9	7014	8661	10699	11064	5825
10	6224	16051	8968	18593	2894
11	204	1000	525	1000	416

Table B.8: Discrete bacteria concentrations 2/11/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	96398	118050	111877	127000	16074
2	6341	2038	12572	7499	1655
3	3699	5248	5644	9849	1844
4	2730	2038	4418	5201	1352
5	2318	3087	5633	4157	2539
6	2730	5201	6301	6305	625
7	2417	2038	5127	4157	525
8	3100	< 100	6006	2038	743

Table B.9: Discrete bacteria concentrations 2/18/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	636	1009	1318	3087	1589
2	1231	1009	2267	2038	4202
3	736	100	1803	100	1527
4	1106	1001	1622	1001	1085
5	985	1009	2501	1009	1722
6	1219	100	1740	100	1444
7	985	2038	1740	3087	625
8	412	100	1085	100	1655
9	630	1009	976	1009	1096
10	630	100	2000	2038	743
11	416	100	1473	2021	976

Table B.10: Discrete bacteria concentrations 3/13/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	79266	113762	149170	125724	111877
2	20553	26623	37788	31784	12712
3	17721	16216	24146	20212	16557
4	13385	15889	15483	18593	4295
5	10044	15731	12572	15731	6739
6	10032	7233	11531	9489	4236
7	6667	7431	8940	7431	3746
8	7163	4157	8211	6362	3159
9	2318	1009	3506	6248	2699
10	2144	1001	3557	1001	2243

Table B.11: Discrete bacteria concentrations 3/26/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	10341	11063	14489	16216	8574
2	4642	5248	6873	9756	3941
3	3159	5201	5207	9756	2144
4	12572	12187	17030	16051	3930
5	10040	7431	17665	9756	4660
6	7666	8661	10670	14881	4354
7	6539	6362	9757	7499	2860
8	42228	56445	47220	61130	3746

Table B.12: Discrete bacteria concentrations 4/02/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	103582	97671	208291	237631	33868
2	17812	15889	84393	59816	12256
3	16010	13578	30442	41443	11591
4	33868	31003	66296	83424	26280
5	19665	25428	24146	42946	170985

Table B.13: Discrete bacteria concentrations 5/08/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	22695	30628	79266	69415	12000
2	35764	43551	84393	71459	17127
3	22695	34168	66296	61898	16010
4	35764	59267	84393	94935	16156
5	121685	116565	655460	161561	55876
6	111877	138106	170985	142498	111877
7	55876	75050	133691	126000	32095
8	66296	72287	90061	100398	27444
9	55876	71459	121685	103271	52820
10	655460	197739	655460	422309	655460

Table B.14: Discrete bacteria concentrations 5/14/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	111877	89683	655460	176660	208291
2	49938	36028	133691	60065	655460
3	30442	53760	74584	89954	655460
4	37788	47505	66296	83820	208291
5	24909	28941	52820	50408	170985
6	39941	56331	84393	83424	208291
7	33868	26313	79266	59045	133691

Table B.15: Discrete bacteria concentrations 6/04/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	1000:1 dilution
1	74584	66120	208291	190349	80178
2	52820	55429	170985	105968	50408
3	70280	55592	170985	86764	33191
4	47220	64172	655460	140377	51272
5	33868	52027	96398	89954	30263
6	55876	57269	103582	109535	32774
7	59124	79385	90061	128362	18593
8	49938	75050	90061	97671	16051
9	74584	80671	121685	112690	30263
10	70280	72079	133691	138481	17402
11	59124	72920	74584	117275	20212
12	55876	59045	74584	116565	21914

Table B.16: Discrete bacteria concentrations 7/17/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	1000:1 dilution
1	18341	10095	133691	123070	6193
2	13385	10095	103582	109585	3087
3	15655	100	70280	41565	5248
4	39941	86616	170985	174033	174035
5	17665	100	111877	52483	3087
6	17721	30871	111877	98499	3087
7	12572	20209	103582	109585	15424
8	6739	100	103582	41202	7431
9	62589	86616	655460	653996	7499
10	3650	100	49938	30606	3087

Table B.17: Discrete bacteria concentrations 7/25/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	5203	1009	55876	51337	1444
2	4107	4157	59124	32774	4418
3	3461	2038	74584	30855	1444
4	3899	2038	62589	36054	2099
5	4712	3087	70280	46636	3277
6	4819	3087	62589	21210	6491

Table B.18: Discrete bacteria concentrations 7/25/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	9185	8502	208291	274742	10314
2	32095	43551	655460	213775	17212
3	27472	29562	655460	167787	27472
4	22247	42946	170985	211157	19665
5	59124	40503	208291	162742	19773
6	52820	76660	655460	472242	32095
7	55876	49519	655460	422309	28903
8	66296	136259	655460	499435	66296
9	84393	78887	655460	338687	59124
10	121685	97575	655460	399458	79266
11	90061	103411	655460	377906	90061
12	74584	89954	655460	338687	90061

Table B.19: Discrete bacteria concentrations 8/28/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	13626	13444	96398	73526	2827
2	21376	14732	111877	65391	2730
3	15483	17224	79266	55429	8342
4	14977	12070	103582	144890	9506
5	49938	56445	170985	149771	13460
6	24146	21914	133691	118900	19665
7	52820	52885	133691	144890	21701
8	24909	25716	655460	197739	44652
9	17721	20432	90061	76660	32095
10	24909	18593	79266	85375	16557
11	35764	27624	103582	78887	15846
12	27444	38465	90061	109535	23762

Table B.20: Discrete bacteria concentrations 9/07/2009 storm

Sample Number	*E. coli* (MPN / 100 ml)	*E. coli* (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	fecal coliform (MPN / 100 ml)	enterococci (MPN / 100 ml)
	100:1 dilution	1000:1 dilution	100:1 dilution	1000:1 dilution	100:1 dilution
1	17127	12070	208291	304423	2991
2	13626	18401	170985	222482	5644
3	33868	51202	208291	138106	10918
4	21701	33191	133691	154838	21701
5	18341	18401	84393	92077	7986
6	16273	9666	55876	72920	8574
7	22695	17585	111877	106197	18333
8	12000	17402	103582	83007	14702
9	9762	5201	33868	27304	4144
10	9493	9849	37788	41074	10036
11	20772	6362	42228	26011	13626
12	17721	20212	47220	43239	18323
13	14461	15889	29985	46420	12087
14	24909	24172	33868	49643	15346
15	30442	35568	47220	63748	27444
16	17127	13313	39941	31975	26280
17	4619	12187	16557	27624	11136
18	5633	18990	35764	50307	10953

C. Appendix: Watershed Rainfall Data and Manipulation

Rainfall in the Raleigh, NC, watershed (Chapters 2 and 3) was monitored using a Davis tipping bucket rain gage with a HOBO event logger. Since tipping bucket rain gages are known to under predict total precipitation depth, a manual rain gage was placed onsite to verify tipping bucket readings and to adjust tipping bucket data as needed. Correction factors were developed for each storm event to relate total rainfall depth between the two data sets. Figure C.1 shows tipping bucket and manual rainfall depths for each storm event. Table C.1 presents tipping bucket and manual rainfall depths along with correction factors.

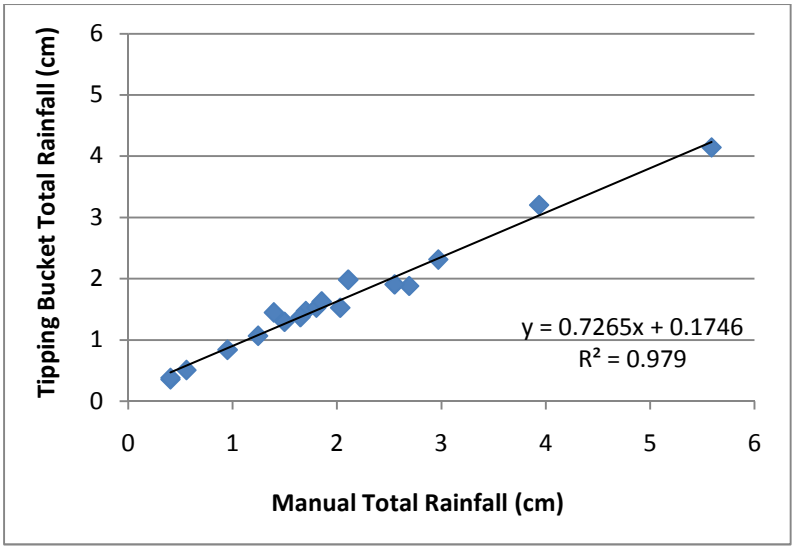

Figure C.1: Tipping bucket rainfall total vs. manual rainfall total

Manual rainfall data were used in analyses when available. However, there were two rainfall events when no manual data were available. Thus, the mean correction factor for all other rainfall events was used to correct tipping bucket rainfall depths for those two events. Further, manual rain gages do not provide a hyetograph for each event, making rainfall intensity calculations impossible. Thus, storm specific correction factors were used to adjust rainfall intensities calculated from tipping bucket data. When a correction factor was unavailable due to missing manual rainfall data, the mean tipping bucket correction factor for all storms was used.

Table C.1: Rainfall data for Raleigh, NC, watershed study

Date	Manual (cm)	Tipping Bucket (cm)	Correction Factor
10/17/2008	2.03	1.52	1.33
11/4/2008	2.69	1.88	1.43
11/14/2008	-	2.36	-
11/25/2008	0.95	0.84	1.14
12/20/2008	2.97	2.31	1.29
1/6/2009	2.55	1.91	1.34
1/28/2009	1.24	1.07	1.17
2/11/2009	0.41	0.36	1.14
2/18/2009	1.70	1.47	1.16
3/13/2009	2.11	1.98	1.06
3/26/2009	1.80	1.52	1.18
4/2/2009	-	0.79	-
5/8/2009	1.85	1.63	1.14
5/14/2009	0.56	0.51	1.10
6/4/2009	3.94	3.20	1.23
7/17/2009	1.40	1.45	0.96
7/25/2009	0.41	0.38	1.07
8/5/2009	1.65	1.37	1.20
8/28/2009	1.50	1.30	1.16
9/7/2009	5.59	4.14	1.35
		Mean =	1.19

D. Appendix: Verification of Bacterial Analysis Method

All bacteria analyses for fecal coliform and *E. coli* in Chapters 2 and 3 followed a modified Colilert (defined substrate technologies; IDEXX, Westbrook, Maine) methodology. The typical Colilert methodology involves incubation at a temperature of 35^{o}C for 24 hours to enumerate total coliform and *E. coli*. However, a study by Yakub et al. (2002) showed that a modified incubation temperature of 44.5^{o}C allowed enumeration of fecal coliform and *E. coli*, fecal coliform being a more desirable indicator bacteria than total coliform. Thus, this modified methodology was employed. Typical application of this modified methodology in such studies as Krometis et al. (2009) involved incubation at 37^{o}C for 4 hours, followed by incubation at 44.5^{o}C for the remaining 20 hours. Incubation at 37^{o}C allows resuscitation of bacteria prior to increasing temperatures to 44.5^{o}C.

In Chapters 2 and 3, the modified methodology consisted of incubation for only 1 to 3 hours at 37^{o}C prior to incubation at 44.5^{o}C for the remaining 24 hours. At the onset of the study, only one incubator was available for use. During this period, samples were incubated at 37^{o}C for 1 to 2 hours before changing the temperature on the incubator to 44.5^{o}C. The incubator reached the target temperature with the samples still inside. Once two incubators became available, samples were incubated in one incubator set to 37^{o}C before being removed and placed into the second incubator set at 44.5^{o}C (which is standard procedure). Thus, some quality control and assurance measures were desired to ensure the validity of this methodology due to the varied incubation time at 37^{o}C and use of only one incubator at the beginning of the study.

An experiment was developed to validate the methodology utilized in Chapters 2 and 3. Eighteen samples were collected throughout a storm event at the watershed studied in Raleigh, NC, on 9/7/2009. Three of these samples (numbers 1, 9, and 18) were utilized to validate three analysis methodologies. The length of time the samples were incubated at a temperature of 37^{o}C prior to incubation at 44.5^{o}C was varied in each methodology. All analyses were run for two dilution factors. The methodologies were:

- Method 1: Incubate at 37^{o}C for 2 hours followed by incubation at 44.5^{o}C for 22 hours.

- Method 2: Incubate at 37^{o}C for 1 hour followed by changing the incubator temperature to 44.5^{o}C for 23 hours (this method involved the use of only one incubator).
- Method 3: Incubate at 37^{o}C for 4 hours followed by incubation at 44.5^{o}C for 20 hours.

The results of these analyses are presented in Table D.1. A maximum reporting limit was reached on the 100:1 dilution of methodology 2 for fecal coliform in sample 1, making statistical analyses involving this sample impossible. However, a 1000:1 dilution was most often utilized for fecal coliform for the data in Chapter 2 and 3.

Table D.1: Results of analysis on enumeration methodologies

Sample	**1**	**9**	**18**	**1**	**9**	**18**	**1**	**9**	**18**
Method	1	1	1	2	2	2	3	3	3
100: dilution									
***E. coli* (MPN/100 ml)**	17127	9762	5633	15292	8712	7720	11269	8211	6739
fecal coliform (MPN/100 ml)	208291	33868	35764	655460[1]	30442	49938	133691	19034	30442
1000:1 dilution									
***E. coli* (MPN/100 ml)**	12070	5201	18990	18593	9849	9756	14881	8661	3087
fecal coliform (MPN/100 ml)	304423	27304	50307	286787	51202	34606	156551	22671	27952

1: Maximum detection limit

Statistical analyses were performed to determine differences among the methodologies. The results are presented in Table D.2. Wilcoxon signed rank tests were used to perform comparisons among the enumeration methodologies using data from corresponding sample numbers as paired observations. The statistical analyses did not reject the null hypothesis that the methodologies are the same ($p < 0.05$).

Table D.2: Statistical analysis of enumeration methodologies

Indicator Bacteria	Dilution	Wilcoxon Signed Rank (p-values)		
		Pair of Methodologies Evaluated		
		1 - 2	*1 - 3*	*2 - 3*
E. coli	100	1.00	0.50	0.25
fecal coliform	100	-	0.25	-
E. coli	1000	1.00	1.00	0.25
fecal coliform	1000	1.00	0.25	0.25

E. Appendix: Hydrologic, Rainfall, and Climate Data for Raleigh, NC, Watershed

Table E.1: Flow and rainfall characteristics for all storms monitored in Raleigh, NC, watershed

Date	Flow Duration (hr)	Volume (L)	Peak Flow (L/s)	Average Flow (L/s)	Rainfall Duration (hr)	Dry Period (hr)	Period Since 0.5 cm (hr)	Average Rainfall Intensity (cm/hr)	Maximum 5 Minute Intensity (cm/hr)
10/17/2008	9.5	116584.9	20.0	3.3	14.1	166.4	396.9	0.14	0.81
11/4/2008	25.0	120113.2	7.3	1.3	28.5	563.2	990.5	0.09	0.87
11/14/2008	27.5	290068.3	48.5	2.9	36.3	13.2	222.0	0.08	4.00
11/25/2008	5.4	31963.6	12.3	1.5	8.6	81.7	216.0	0.11	0.69
12/20/2008	18.3	147455.8	43.4	2.8	23.8	80.5	204.5	0.13	3.53
1/6/2009	14.6	122672.0	18.7	2.5	12.9	39.4	39.4	0.20	2.04
1/28/2009	2.6	78513.5	54.4	7.4	2.3	9.4	529.3	0.55	8.89
2/11/2009	1.0	24057.2	22.8	6.1	0.3	209.5	209.5	1.43	3.48
2/18/2009	7.0	97977.6	15.7	3.7	7.3	152.0	361.7	0.23	0.70
3/13/2009	10.5	145176.4	18.0	3.8	12.7	230.1	253.7	0.17	1.30
3/26/2009	3.4	48767.5	31.3	3.8	27.9	137.8	137.8	0.06	5.77
4/2/2009	2.9	24569.5	16.1	2.2	7.8	72.0	86.3	0.12	2.18
5/8/2009	1.7	78216.5	49.1	11.2	0.8	17.1	50.1	2.35	5.91
5/14/2009	1.3	27197.9	32.9	5.2	1.4	73.5	158.1	0.41	3.35
6/4/2009	2.6	153105.4	59.0	14.8	10.1	156.7	432.3	0.39	3.37
7/17/2009	2.6	75769.3	142.8	7.6	11.0	25.3	100.5	0.13	7.35
7/25/2009	0.7	18593.0	27.5	6.4	1.9	34.5	34.5	0.22	2.93
8/5/2009	4.8	78765.5	85.7	4.3	6.8	9.4	50.2	0.24	6.60
8/28/2009	3.6	88563.3	34.3	6.5	9.2	92.0	540.3	0.16	2.82
9/7/2009	8.2	288227.4	45.8	9.3	20.2	156.0	223.8	0.28	4.53

Table E.2: Average climate conditions – 1 day preceding rainfall event for all storms monitored in Raleigh, NC, watershed

Date	Air Temperature (^{o}C)	Relative Humidity (%)	Vapor Pressure (mb)	Soil Moisture (m^3/m^3)	Solar Radiation (W / m^2)	Potential Evapotranspiration (cm)
10/17/2008	18.4	85.3	207.8	0.201	37.7	0.33
11/4/2008	14.0	85.6	139.0	0.206	17.4	0.29
11/14/2008	13.5	95.4	158.7	0.223	37.8	0.10
11/25/2008	3.8	59.7	32.0	0.225	79.5	0.12
12/20/2008	16.6	84.4	171.3	0.256	31.8	0.20
1/6/2009	13.5	93.9	153.3	0.274	30.1	0.07
1/28/2009	9.6	93.3	114.3	0.264	32.4	0.07
2/11/2009	16.9	71.8	138.8	0.240	126.2	0.30
2/18/2009	3.8	47.4	24.0	0.223	193.3	0.12
3/13/2009	9.4	37.3	29.4	0.233	145.3	0.40
3/26/2009	6.7	44.5	30.4	0.250	53.9	0.31
4/2/2009	16.2	90.0	181.8	0.267	50.4	0.18
5/8/2009	22.7	74.8	240.5	0.152	263.0	0.38
5/14/2009	18.4	74.5	171.5	0.201	262.7	0.45
6/4/2009	24.3	74.6	268.4	0.172	167.1	0.62
7/17/2009	25.7	81.6	335.3	0.131	213.7	0.41
7/25/2009	25.6	76.8	302.1	0.216	272.5	0.49
8/5/2009	25.7	80.6	334.3	0.211	217.2	0.46
8/28/2009	25.5	79.0	318.0	0.194	160.5	0.50
9/7/2009	23.0	70.3	219.7	0.189	164.4	0.45

Table E.3: Average climate conditions – 2 days preceding rainfall event for all storms monitored in Raleigh, NC, watershed

Date	Rainfall Total (cm)	Air Temperature (^{o}C)	Relative Humidity (%)	Vapor Pressure (mb)	Soil Moisture (m^3/m^3)	Solar Radiation (W / m^2)	Potential Evapotranspiration (cm)
10/17/2008	0.00	19.6	80.8	207.2	0.202	108.3	0.32
11/4/2008	0.00	14.1	74.9	113.3	0.207	94.6	0.28
11/14/2008	0.23	12.3	79.3	115.3	0.224	76.2	0.14
11/25/2008	0.00	3.5	57.9	28.6	0.226	110.2	0.14
12/20/2008	0.10	14.9	90.8	164.0	0.256	38.9	0.12
1/6/2009	1.12	11.6	94.9	134.9	0.272	31.3	0.06
1/28/2009	0.36	7.2	82.1	80.8	0.263	27.5	0.09
2/11/2009	0.00	15.1	70.8	120.5	0.242	120.5	0.25
2/18/2009	0.03	2.9	50.8	24.5	0.224	162.3	0.15
3/13/2009	0.00	14.3	45.5	79.8	0.236	148.3	0.39
3/26/2009	0.00	7.7	48.6	37.1	0.253	145.8	0.29
4/2/2009	0.05	15.3	79.4	145.2	0.270	74.9	0.25
5/8/2009	0.15	22.2	79.0	247.2	0.151	226.9	0.26
5/14/2009	0.00	17.7	69.1	143.0	0.202	270.4	0.44
6/4/2009	0.00	24.8	72.3	267.7	0.179	205.1	0.62
7/17/2009	0.10	25.7	77.5	315.3	0.131	195.5	0.44
7/25/2009	1.07	25.4	76.0	292.0	0.204	265.7	0.47
8/5/2009	0.38	25.8	78.4	318.0	0.212	236.7	0.46
8/28/2009	0.00	26.2	75.7	310.8	0.196	198.9	0.46
9/7/2009	0.00	23.4	68.6	219.5	0.191	189.8	0.41

Table E.4: Average climate conditions – 7 days preceding rainfall event for all storms monitored in Raleigh, NC, watershed

Date	Rainfall Total (cm)	Air Temperature (^{o}C)	Relative Humidity (%)	Vapor Pressure (mb)	Soil Moisture (m^3/m^3)	Solar Radiation (W / m^2)	Potential Evapotranspiration (cm)
10/17/2008	0.15	19.1	77.2	185.4	0.207	149.5	0.28
11/4/2008	0.00	9.2	66.5	66.1	0.213	140.2	0.26
11/14/2008	0.41	11.9	73.3	100.8	0.234	123.0	0.21
11/25/2008	0.10	3.0	52.4	25.7	0.233	125.7	0.17
12/20/2008	0.30	11.6	88.4	129.5	0.263	43.8	0.10
1/6/2009	1.30	6.7	69.5	66.5	0.260	82.3	0.14
1/28/2009	0.53	4.4	64.6	47.3	0.274	77.2	0.14
2/11/2009	0.00	8.8	55.8	63.3	0.252	159.5	0.24
2/18/2009	0.38	9.2	52.4	53.3	0.230	153.8	0.27
3/13/2009	0.00	16.7	51.9	94.9	0.250	199.2	0.47
3/26/2009	1.70	9.3	56.6	53.3	0.269	212.1	0.29
4/2/2009	4.98	15.2	77.0	144.1	0.311	131.0	0.27
5/8/2009	5.61	21.6	78.4	233.1	0.153	189.6	0.41
5/14/2009	2.36	19.0	74.0	180.9	0.193	225.7	0.43
6/4/2009	0.25	24.0	70.7	244.3	0.200	263.9	0.54
7/17/2009	0.91	24.7	73.9	272.4	0.128	210.1	0.46
7/25/2009	1.47	24.5	74.7	272.0	0.172	229.1	0.43
8/5/2009	5.23	25.6	82.1	336.2	0.218	189.4	0.42
8/28/2009	0.53	25.1	80.6	312.7	0.200	174.7	0.36
9/7/2009	0.38	21.3	70.4	194.2	0.197	192.8	0.39

Table E.5: Average climate conditions – 14 days preceding rainfall event for all storms monitored in Raleigh, NC, watershed

Date	Rainfall Total (cm)	Air Temperature (^{o}C)	Relative Humidity (%)	Vapor Pressure (mb)	Soil Moisture (m^3/m^3)	Solar Radiation (W / m^2)	Potential Evapotranspiration (cm)
10/17/2008	0.41	18.7	78.7	185.7	0.216	154.4	0.27
11/4/2008	0.25	10.9	69.9	87.3	0.221	140.6	0.23
11/14/2008	2.31	12.3	74.3	105.1	0.229	119.9	0.26
11/25/2008	2.72	6.9	63.9	65.8	0.239	108.3	0.17
12/20/2008	4.04	10.1	79.2	112.9	0.268	63.4	0.11
1/6/2009	2.46	8.5	71.2	82.7	0.264	76.9	0.14
1/28/2009	1.27	1.5	63.8	36.5	0.260	91.6	0.12
2/11/2009	0.71	6.6	57.0	49.4	0.260	157.0	0.23
2/18/2009	0.38	8.1	53.0	52.2	0.243	160.2	0.26
3/13/2009	5.00	9.9	62.6	71.7	0.272	162.2	0.32
3/26/2009	6.73	8.8	68.5	65.0	0.274	147.3	0.24
4/2/2009	6.88	12.2	67.9	100.3	0.289	164.3	0.28
5/8/2009	5.61	21.5	72.4	205.7	0.167	232.4	0.49
5/14/2009	7.98	20.3	76.2	206.2	0.173	208.1	0.42
6/4/2009	1.14	23.1	75.7	249.8	0.207	235.8	0.47
7/17/2009	1.07	24.3	71.7	254.4	0.126	205.7	0.44
7/25/2009	3.89	24.6	75.0	275.4	0.152	218.9	0.45
8/5/2009	7.11	25.6	80.3	324.3	0.215	206.4	0.45
8/28/2009	0.97	25.6	80.2	324.4	0.200	183.6	0.39
9/7/2009	1.91	23.3	73.8	246.9	0.196	193.4	0.39

Table E.6: Average climate conditions – 28 days preceding rainfall event for all storms monitored in Raleigh, NC, watershed

Date	Rainfall Total (cm)	Air Temperature (^{o}C)	Relative Humidity (%)	Vapor Pressure (mb)	Soil Moisture (m^3/m^3)	Solar Radiation (W / m^2)	Potential Evapotranspiration (cm)
10/17/2008	4.24	18.8	78.5	189.3	0.225	153.8	0.29
11/4/2008	2.18	14.6	74.1	133.5	0.219	147.8	0.24
11/14/2008	4.09	12.5	73.6	109.0	0.226	129.1	0.24
11/25/2008	4.80	9.2	68.3	81.3	0.233	119.2	0.23
12/20/2008	7.37	7.8	73.1	80.5	0.255	77.5	0.14
1/6/2009	8.76	9.5	75.8	100.8	0.271	69.6	0.13
1/28/2009	5.11	3.9	67.7	51.6	0.269	82.7	0.13
2/11/2009	3.05	4.1	60.8	43.9	0.260	123.6	0.17
2/18/2009	3.07	6.3	57.9	47.8	0.257	136.4	0.22
3/13/2009	6.63	7.7	57.4	54.5	0.257	163.3	0.27
3/26/2009	11.73	9.2	66.2	68.4	0.274	160.9	0.27
4/2/2009	11.91	12.2	68.3	94.3	0.279	154.5	0.29
5/8/2009	7.47	18.2	67.1	153.0	0.198	221.9	0.44
5/14/2009	8.64	19.3	68.3	169.5	0.187	242.4	0.47
6/4/2009	5.79	20.8	74.5	213.5	0.209	232.8	0.45
7/17/2009	1.37	25.1	68.0	251.7	0.152	249.1	0.51
7/25/2009	4.34	24.6	70.1	251.1	0.141	232.4	0.47
8/5/2009	9.98	24.9	76.9	292.8	0.177	208.8	0.45
8/28/2009	6.93	25.7	79.7	322.8	0.205	188.3	0.40
9/7/2009	3.10	24.5	77.4	288.4	0.199	183.5	0.39

F. Appendix: Raw data from Charlotte, NC, stormwater control measures

Table F.1: Dry Detention 1 – raw data

Date	fecal coliform		*E. coli*	
	inlet	outlet	inlet	outlet
2/25/2005	3800	5000	> 2400	> 2400
3/23/2005	120	380	140	150
4/11/2005	< 100	990	80	980
6/1/2005	15000	20000	> 2400	> 2400
10/6/2005	42000	53000	> 2400	> 2400
12/5/2005	2100	2100	> 2400	1700
12/16/2005	270	330	210	240
3/22/2006	1400	2100	> 2400	1400
7/6/2006	21000	4600	2400	> 2400

Table F.2: Dry Detention 2 – raw data

Date	fecal coliform		*E. coli*	
	inlet	outlet	inlet	outlet
1/14/2005	360	450	140	340
2/14/2005	630	360	50	40
3/24/2005	120	310	200	230
4/7/2005	5400	2500	> 2400	> 2400
4/13/2005	690	2000	1100	1400
6/1/2005	15000	17000	> 2400	2400
8/23/2005	6000	> 6000	2400	1300
10/6/2005	12000	> 20000	> 2400	> 2400
11/22/2005	2400	4000	1600	630
12/5/2005	630	690	370	730
12/16/2005	1500	420	1600	490
12/29/2005	120	440	130	370

Table F.3: Wet Pond – raw data

Date	fecal coliform		*E. coli*	
	inlet	outlet	inlet	outlet
8/12/2004	91000	50000		
9/27/2004	73000	270		
10/13/2004	42000	10000		
11/29/2004	6600	7000	> 2400	> 2400
12/6/2004	15000	90	> 2400	80
1/14/2005	3100	5900		
2/14/2005	1400	1600	1200	2000
3/23/2005	8200	4800	> 2400	2400
4/12/2005	6700	540	> 2400	1100
5/11/2005	20000	28000	> 2400	> 2400
6/1/2005	3700	7800	> 2400	> 2400
12/16/2005	4200	4200	> 2400	> 2400
3/22/2006	1400	1100	1400	1100
4/26/2006	8400	530	2400	270

Table F.4: Wetland 1 – raw data

Date	fecal coliform		*E. coli*	
	inlet	outlet	inlet	outlet
3/16/2004	4400	90		
3/30/2004	1400	< 100		
9/29/2004	5800	180		
12/9/2004	61000	270	> 2400	20
12/10/2004	6900	180	> 2400	170
2/25/2005	84000	< 100	> 2400	30
3/23/2005	5800	250	2400	210
4/13/2005	7000	500	> 2400	550
6/1/2005	13000	250	> 2400	120

Table F.5: Wetland 2 – raw data

Date	fecal coliform		*E. coli*	
	inlet	outlet	inlet	outlet
9/7/2004	234873	22000		
9/27/2004	41632	4600		
10/13/2004	56583	7900		
11/4/2004	17096	8100		
12/9/2004	7271	11000	> 2400	> 2400
1/14/2005	7064	4200	> 2400	2400
2/14/2005	< 100	< 100	114	< 10
2/25/2005	37474	3100	1927	1600
3/8/2005	4306	3200	725	1300
4/13/2005	2937	6900	5729	2400
6/28/2005	44573	29000		
10/6/2005	55581	50000	1676	2400
12/5/2005	2443	2100	2400	2200
12/16/2005	3190	560	2307	390
12/29/2005	290	190	272	390

Table F.6: Bioretention – raw data

Date	fecal coliform		*E. coli*	
	inlet	outlet	inlet	outlet
8/12/2004	77000	1500		
8/28/2004	7500	< 100		
9/27/2004	14000	< 100		
10/13/2004	20000	22000		
11/4/2004	35000	180		
12/6/2004	1100	< 100	1200	< 1
1/14/2005	540	< 100	820	30
2/14/2005	< 100	< 100	< 10	< 10
2/22/2005	< 100	< 100	120	< 10
3/8/2005	230	< 100	120	< 10
4/7/2005	3100	< 100	> 2400	< 1
4/13/2005	2700	120	1400	70
5/13/2005	> 60000	> 60000	> 2400	> 2400
6/28/2005	50000	1100	> 2400	1200
10/6/2005	5000	400	48	10
12/5/2005	1900	190	2000	30
12/16/2005	380	19	10	30
12/29/2005	< 100	< 100	4	1
3/22/2006	280	< 100	210	28

Table F.7: Proprietary 1 – raw data

Date	fecal coliform		*E. coli*	
	inlet	outlet	inlet	outlet
10/6/2005	190	63	53	26
12/5/2005	100	100	1	2
4/19/2006	8600	6100	2000	2400
4/26/2006	100	100	170	10
9/1/2006	22000	490	13	33
9/14/2006	200	200	15	35
10/17/2006	820	330	21	60

Table F.8: Proprietary 2 – raw data

Date	fecal coliform		*E. coli*	
	inlet	outlet	inlet	outlet
10/6/2005	690	1300	58	41
12/5/2005	100	100	1	1
4/19/2006	100	100	1	13
9/1/2006	490	2900	2	29
9/14/2006	250	330	27	170
10/18/2006	200	200	1	3

Table F.9: Proprietary 3 – raw data

Date	fecal coliform		*E. coli*	
	inlet	outlet	inlet	outlet
10/6/2005	750	750	29	16
12/5/2005	1100	1100	290	290
4/19/2006	2400	5000	1700	2400
9/1/2006	3000	4400	81	38
9/14/2006	5200	50000	490	2400
10/17/2006	330	200	66	55

G. Appendix: Raw data from Wilmington, NC, stormwater control measures

Table G.1: Wet Pond 1 – raw data

Date	*E. coli*		enterococci	
	inlet	outlet	inlet	outlet
6/23/2008	988	148	5475	10462
8/13/2008	2851	31	504	63
8/27/2008	> 24196	19863	> 24196	> 24196
9/25/2008	6310	41	> 24196	388
1/13/2009	4839	40	4,839	2
2/18/2009	651	< 2	330	13
4/2/2009	403	2	> 4839	83
5/14/2009	255	40	278	12
8/12/2009	15531	521	496	62
8/14/2009	1226	387	6940	3106
9/22/2009	5794	731	344	234
10/5/2009	3466	8	2098	168
11/10/2009	4611	2	12997	3973
11/11/2009	6488	19863	10112	4374
2/2/2010	1633	8	1314	32
2/9/2010	2092	1633	2599	2755

Table G.2: Wet Pond 2 – raw data

Date	*E. coli*		enterococci	
	inlet	outlet	inlet	outlet
2/18/2008	2909	697	8664	1483
2/22/2008	< 10	520	52	20
3/7/2008	3130	10	160	20
6/23/2008	613	399	364	97
8/13/2008	3649	31	31	< 10
8/27/2008	> 24196	521	> 24196	1240
9/25/2008	8840	< 10	1414	< 10
1/13/2009	1095	< 10	134	< 8
2/18/2009	3106	6	88	< 2
4/2/2009	582	6	2	4
5/14/2009	3106	1373	2240	1633
8/12/2009	81640	24	242	120
8/14/2009	192	120	197	49
9/22/2009	2068	3466	97	870
10/5/2009	90	8	27	< 2
11/10/2009	> 24196	172	11199	159
11/11/2009	19863	14136	1212	725
2/2/2010	55	< 2	150	< 2
2/9/2010	74	37	245	6

Table G.3: Wetland 1 – raw data

Date	*E. coli*		enterococci	
	inlet	outlet	inlet	outlet
2/18/2008	785	1017	> 24196	1657
2/22/2008	697	41	467	146
3/7/2008	697	61	723	30
6/23/2008	75	752	12033	29090
8/13/2008	2760	311	61	< 10
8/27/2008	9210	10500	8660	> 24196
9/25/2008	14136	2909	4360	3609
1/13/2009	870	3973	2407	1632
2/18/2009	158	51	225	14
4/2/2009	1160	303	135	21
5/14/2009	1540	1317	821	284
8/12/2009	403	1633	86	139
8/14/2009	3973	1842	6870	5640
9/22/2009	409	9804	1373	333
10/5/2009	259	36540	1540	2755
11/10/2009	6488	> 24196	2827	1785
11/11/2009	2613	6488	935	957
2/2/2010	75	49	74	< 2
2/9/2010	182	17	387	15

Table G.4: Wetland 2 – raw data

Date	*E. coli*		enterococci	
	inlet	outlet	inlet	outlet
1/17/2008	-	-	866	172
2/18/2008	256	199	1892	1594
2/22/2008	41	41	738	1198
3/7/2008	160	317	842	512
6/23/2008	771	723	1350	3950
8/13/2008	< 10	52	201	< 10
8/27/2008	5790	3870	> 24196	> 24196
9/25/2008	323	323	4360	2500
1/13/2009	731	449	2599	449
2/18/2009	2599	731	250	86
4/2/2009	2240	110	3973	22
5/14/2009	456	76	227	29
8/12/2009	690	11199	166	108
8/14/2009	403	521	3973	8350
9/22/2009	2092	4839	690	690
10/5/2009	651	11199	241	2105
11/10/2009	9804	> 24196	1373	9804
11/11/2009	960	933	250	448
2/2/2010	26	6	140	12
2/9/2010	46	279	182	1842

Table G.5: Bioretention – raw data

Date	*E. coli*			enterococci		
	inlet	outlet - Bioretenion-S	outlet - Bioretention-D	inlet	outlet - Bioretenion-S	outlet - Bioretention-D
2/22/2008	203	3255	< 10	591	2187	31
3/72008	10	1043	< 10	75	279	< 10
6/23/2008	1187	12033	8164	983	249	134
8/13/2008	135	384	< 10	328	480	< 10
8/27/2008	< 10	213	< 10	552	3870	121
9/25/2008	52	1350	< 10	638	2310	52
11/3/2008	108	< 10	638	119	20	389
11/13/2008	3433	19863	211	30	1223	20
1/13/2009	42	137	6	197	437	4
2/18/2009	< 2	6	10	225	32	10
4/2/2009	52	43	< 2	> 4839	30	12
5/14/2009	44	1095	3973	75	3466	118
8/12/2009	275	3106	81	99	92	58
8/14/2009	3466	14136	2	4210	3185	80
9/22/2009	7701	6867	731	247	218	92
10/5/2009	137	10	284	922	106	253
11/10/2009	1961	< 2	821	2382	4839	1454
11/11/2009	4884	1178	< 10	1174	605	< 10
2/2/2010	4	8	< 2	582	65	< 2
2/9/2010	4	6	< 2	66	121	22

H. Appendix: Wilmington Bioretention – Additional Data

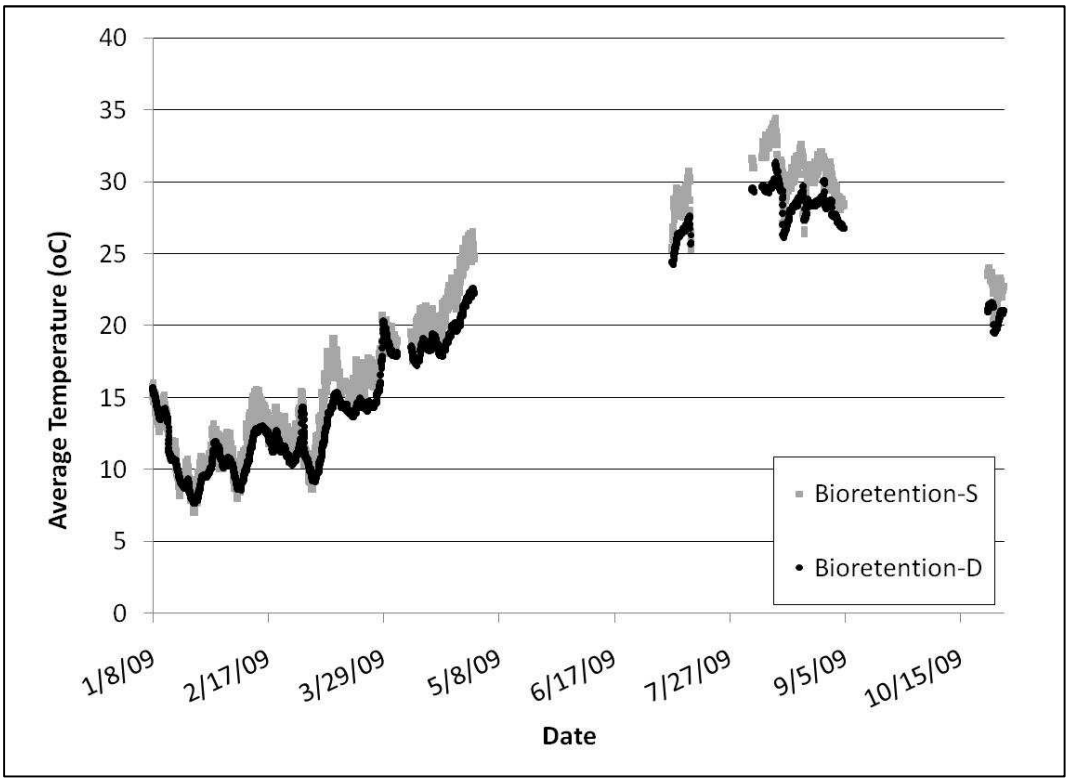

Figure H.1: Average hourly temperature in Bioretention-S and Bioretention-D

Table H.1: Soil bacteria analysis results – 12/15/2008

Location		Core	Depth (in)	*E. coli* (MPN / 20 g)	enterococci (MPN / 20 g)
Bioretention-S	Site 1	Core 1 A	0 - 7	>2419.6	>2419.6
		Core 1 B	0 - 7	2419.6	>2419.6
		Core 2 A	7 - 12	1413.6	>2419.6
		Core 2 B	7 - 12	1732.9	>2419.6
	Site 2	Core 1 A	0 - 8.5	118.7	>2419.6
		Core 1 B	0 - 8.5	45.5	920.8
		Core 2 A	8.5 - 9.5	87.8	920.8
		Core 2 B	8.5 - 9.5	14.8	920.8
	Site 3	Core 1 A	0 - 8	2.0	435.2
		Core 1 B	0 - 8	<1.0	325.5
Bioretention-D	Site 1	Core 1 A	0 - 10.5	1.0	>2419.6
		Core 1 B	0 - 10.5	<1.0	>2419.6
		Core 2 A	10.5 - 21	<1.0	>2419.6
		Core 2 B	10.5 - 21	<1.0	2419.6
		Core 3 A	21 - 22.5	<1.0	>2419.6
		Core 3 B	21 - 22.5	<1.0	>2419.6
	Site 2	Core 1 A	0 - 10	5.2	>2419.6
		Core 1 B	0 - 10	4.1	>2419.6
		Core 2 A	10 - 24	<1.0	>2419.6
		Core 2 B	10 - 24	<1.0	629.4
		Core 3 A	24 - 27.5	2.0	>2419.6
		Core 3 B	24 - 27.5	3.0	960.6
	Site 3	Core 1 A	0 - 7	<1.0	>2419.6
		Core 1 B	0 - 7	<1.0	1986.3
		Core 2 A	7 - 13	1.0	1119.9
		Core 2 B	7 - 13	<1.0	>2419.6
		Core 3 A	13 - 17.5	<1.0	>2419.6
		Core 3 B	13 - 17.5	<1.0	>2419.6

Table H.2: Soil bacteria analysis results – 3/5/2009

Location		Core	Depth (in)	*E. coli* (MPN / 20 g)	enterococci (MPN / 20 g)
Bioretention-S	**Site 1**	Core 1 A	0 - 8	5.2	1011.2
		Core 1 B	0 - 8	8.6	1011.2
		Core 2 A	0 - 12	10.9	574.8
		Core 2 B	0 - 12	17.1	960.6
	Site 2	Core 1 A	0 - 8.5	6.3	313
		Core 1 B	0 - 8.5	4.1	250.8
		Core 2 A	X	X	X
		Core 2 B	X	X	X
	Site 3	Core 1 A	0 - 8	<1	1011.2
		Core 1 B	0 - 8	<1	3.1
Bioretention-D	**Site 1**	Core 1 A	0 - 11	5.2	1011.2
		Core 1 B	0 - 11	2	240
		Core 2 A	11 - 18	1	960.6
		Core 2 B	11 - 18	<1	913.9
		Core 3 A	18 - 21	<1	913.9
		Core 3 B	18 - 21	2	330
	Site 2	Core 1 A	0 - 9	5.2	1011.2
		Core 1 B	0 - 9	1	689.3
		Core 2 A	9 - 18	2	665.3
		Core 2 B	9 - 18	13.7	574.8
		Core 3 A	18 - 24	3.1	1011.2
		Core 3 B	18 - 24	24.2	960.6
	Site 3	Core 1 A	0 - 10	1	1011.2
		Core 1 B	0 - 10	17.3	1011.2
		Core 2 A	10 - 19	22.6	1011.2
		Core 2 B	10 - 19	<1	960.6
		Core 3 A	19 - 22	6.3	913.9
		Core 3 B	19 - 22	<1	1011.2

Table H.3: Soil bacteria analysis results – 6/1/2009

Location		Core	Depth (in)	*E. coli* (MPN / 20 g)	enterococci (MPN / 20 g)
Bioretention-S	Site 1	Core 1 A	0 - 10	27.5	>2419.6
		Core 1 B	0 - 10	3.0	>2419.6
		Core 2 A	10 - 12	<1.0	>2419.6
		Core 2 B	10 - 12	2.0	>2419.6
	Site 2	Core 1 A	0 - 9	6.3	>2419.6
		Core 1 B	0 - 9	18.5	435.2
		Core 2 A	X	X	X
		Core 2 B	X	X	X
	Site 3	Core 1 A	0 - 9	<1.0	17.1
		Core 1 B	0 - 9	10.9	86.9
Bioretention-D	Site 1	Core 1 A	0 - 9	83.6	>2419.6
		Core 1 B	0 - 9	24.6	115.9
		Core 2 A	9 - 15	36.4	71.4
		Core 2 B	9 - 15	1.0	33.7
		Core 3 A	15 - 20.5	31.3	45.4
		Core 3 B	15 - 20.5	10.9	34.1
	Site 2	Core 1 A	0 - 10	13.5	43.9
		Core 1 B	0 - 10	9.7	52.8
		Core 2 A	10 - 17	33.2	113.3
		Core 2 B	10 - 17	29.2	168.2
		Core 3 A	17 - 21	18.3	73.3
		Core 3 B	17 - 21	6.3	42.2
	Site 3	Core 1 A	0 - 8	248.9	>2419.6
		Core 1 B	0 - 8	365.4	>2419.6
		Core 2 A	8 - 15	88.4	>2419.6
		Core 2 B	8 - 15	11.9	1966.3
		Core 3 A	X	X	X
		Core 3 B	X	X	X

Table H.4: Soil bacteria analysis results – 8/4/2009

Location		Core	Depth (in)	*E. coli* (MPN / 20 g)	enterococci (MPN / 20 g)
Bioretention-S	Site 1	Core 1 A	0 - 6.5	< 1.0	> 2419.6
		Core 1 B	0 - 6.5	< 1.0	146.4
		Core 2 A	6.5 - 10.5	< 1.0	> 2419.6
		Core 2 B	6.5 - 10.5	1.0	1299.7
	Site 2	Core 1 A	0 - 9	< 1.0	109.5
		Core 1 B	0 - 9	< 1.0	37.3
		Core 2 A	X	X	X
		Core 2 B	X	X	X
	Site 3	Core 1 A	0 - 8.5	1.0	44.6
		Core 1 B	0 - 8.5	< 1.0	172.7
Bioretention-D	Site 1	Core 1 A	0 - 7	< 1.0	64.5
		Core 1 B	0 - 7	< 1.0	214.2
		Core 2 A	7 - 15	1.0	135.4
		Core 2 B	7 - 15	1.0	2419.6
		Core 3 A	15 - 20.5	< 1.0	> 2419.6
		Core 3 B	15 - 20.5	< 1.0	74.1
	Site 2	Core 1 A	0 - 9	2.0	249.5
		Core 1 B	0 - 9	5.2	648.8
		Core 2 A	9 - 15.5	2.0	410.6
		Core 2 B	9 - 15.5	1.0	770.1
		Core 3 A	15.5 - 23.5	3.1	648.8
		Core 3 B	15.5 - 23.5	< 1.0	461.1
	Site 3	Core 1 A	0 - 9.5	< 1.0	> 2419.6
		Core 1 B	0 - 9.5	< 1.0	> 2419.6
		Core 2 A	9.5 - 14	< 1.0	727.0
		Core 2 B	9.5 - 14	< 1.0	344.8
		Core 3 A	X	X	X
		Core 3 B	X	X	X

Table H.5: Wilmington bioretention hydrology data February 2007 – November 2007

Date	Rainfall (in)	Bioretention-S			Bioretention-D		
		Inflow (L)	Outflow (L)	Peak Flow (L/s)	Inflow (L)	Outflow (L)	Peak Flow (L/s)
2/13/2007	0.41	5488	1008	0.113	10369	3025	0.227
2/21/2007	0.13	1381	0	0.000	2569	74	0.017
2/25/2007	0.23	2848	201	0.057	5355	750	0.085
3/1/2007	0.45	6075	793	0.113	11484	2631	0.227
3/16/2007	1.04	14755	3370	0.255	27921	10173	0.453
3/21/2007	0.18	2115	23	0.017	3962	11	0.025
6/5/2007	0.05	208	17	0.028	340	1	0.001
6/20/2007	0.84	11795	2472	0.510	22349	7649	0.878
7/1/2007	0.11	1088	23	0.028	2012	20	0.045
7/5/2007	0.03	46	36	0.005	46	0	0.000
7/7/2007	0.3	3875	224	0.142	7305	263	0.057
7/8/2007	0.01	15	0	0.000	15	0	0.000
7/9/2007	0.03	46	0	0.000	46	9	0.001
7/10/2007	0.13	1381	82	0.028	2569	23	0.014
7/11/2007	0.07	501	0	0.000	897	4	0.001
7/20/2007	0.26	3288	93	0.113	6191	218	0.057
7/26/2007	0.06	355	0	0.000	619	4	0.001
7/28/2007	3.41	50138	26094	24.412	93949	34771	27.555
7/30/2007	1.17	16696	9017	0.878	31543	11130	1.104
8/7/2007	0.18	2115	34	0.028	3962	34	0.057
8/10/2007	0.44	5928	796	0.425	11205	3347	0.736
8/21/2007	0.15	1675	25	0.034	3126	23	0.034
8/26/2007	0.11	1088	31	0.020	2012	11	0.014
8/26/2007	0.1	941	34	0.057	1733	14	0.028
8/27/2007	1.95	28341	10405	1.586	53274	15018	4.106
9/11/2007	0.05	208	17	0.011	340	1	0.011
9/12/2007	0.62	8568	1764	0.821	16220	4744	0.708
9/14/2007	0.22	2701	357	0.255	5076	473	0.142
9/14/2007	0.21	2555	507	0.198	4798	1090	0.283
9/15/2007	0.51	6955	2923	0.821	13155	4155	0.651
9/20/2007	0.76	10622	2387	0.680	20120	6542	0.538
9/22/2007	0.78	10915	4741	1.020	20678	5475	0.821
9/27/2007	0.06	355	0	0.000	619	7	0.011
9/27/2007	0.04	61	0	0.000	61	4	0.003
10/25/2007	1.19	16994	6542	1.812	32100	16771	2.436
10/25/2007	1.02	14456	2849	0.595	27364	31529	1.020
11/15/2007	0.4	5342	88	0.085	10091	3605	0.453

Table H.6: Wilmington bioretention hydrology data December 2007 – May 2009

Date	Rainfall (in)	Bioretention-S			Bioretention-D		
		Inflow (L)	Outflow (L)	Peak Flow (L/s)	Inflow (L)	Outflow (L)	Peak Flow (L/s)
12/15/2007	1.45	20876	8258	1.133	39344	15786	0.963
12/21/2007	0.66	9155	1699	0.198	17334	7547	0.425
12/23/2007	0.05	208	0	0.000	340	28	0.014
12/25/2007	0.53	7248	1569	0.312	13713	5888	0.481
1/11/2008	0.27	3435	442	0.312	6469	884	0.170
1/11/2008	0.5	6808	2212	0.453	12877	5925	0.595
1/13/2008	0.07	501	14	0.008	897	57	0.028
1/17/2008	1.13	16099	6313	0.595	30429	15440	0.595
1/19/2008	0.42	5635	4373	0.255	10648	13013	0.453
1/23/2008	0.05	208	0	0.000	340	65	0.037
1/26/2008	0.015	23	0	0.000	23	470	0.037
1/30/2008	0.013	20	0	0.000	20	190	0.048
2/1/2008	0.33	4315	1042	0.368	8141	3319	0.481
2/12/2008	0.94	13262	5268	0.538	25135	18136	0.963
2/21/2008	0.41	5488	6870	0.396	10369	25089	0.935
10/11/2008	1.8	26101	8935	0.736	49095	16259	0.765
10/18/2008	0.31	4022	4	0.008	7584	425	0.057
10/24/2008	1.01	14307	4826	0.736	27085	7927	0.680
11/3/2008	0.81	11355	1722	0.283	21513	5500	0.396
11/4/2008	0.03	46	0	0.000	46	14	0.007
11/13/2008	1.82	26400	10985	1.020	49652	13078	0.850
11/14/2008	0.35	4608	1461	0.396	8698	2840	0.425
11/15/2008	0.02	31	0	0.000	31	0	0.000
11/29/2008	1.41	20279	4330	0.425	38229	18102	0.680
12/2/2008	0.09	795	1	0.001	1454	144	0.028
12/6/2008	0.04	61	0	0.000	61	0	0.000
12/10/2008	0.09	795	2	0.002	1454	74	0.028
12/10/2008	0.68	9448	3529	0.708	17892	1	0.085
12/11/2008	0.83	11649	7055	0.595	22071	15180	0.793
12/17/2008	0.14	1528	2	0.006	2847	532	0.057
12/20/2008	0.03	46	0	0.000	46	0	0.000
5/7/2009	0.06	355	11	0.028	619	1	0.002
5/11/2009	0.59	8128	1272	0.510	15384	3189	0.991
5/14/2009	0.45	6075	852	0.312	11484	1773	0.312
5/17/2009	2.09	30431	13223	1.529	57174	16312	1.303
5/21/2009	0.02	31	0	0.000	31	0	0.000
5/22/2009	0.04	61	0	0.000	61	0	0.000
5/24/2009	0.09	795	59	1.180	1454	79	0.312

Table H.7: Wilmington bioretention hydrology data June 2009 – December 2009

Date	Rainfall (in)	Bioretention-S			Bioretention-D		
		Inflow (L)	Outflow (L)	Peak Flow (L/s)	Inflow (L)	Outflow (L)	Peak Flow (L/s)
6/5/2009	0.07	501	1	0.000	897	0	0.000
6/9/2009	0.2	2408	3	0.004	4519	42	0.014
7/24/2009	0.78	10915	4273	1.784	20678	5423	1.048
7/31/2009	0.02	31	0	0.000	31	0	0.000
8/6/2009	0.18	2115	110	0.255	3962	365	0.821
8/11/2009	0.09	795	76	0.255	1454	144	0.680
8/12/2009	0.52	7102	1742	0.991	13434	5225	5.494
8/13/2009	0.21	2555	17	0.017	4798	515	0.142
8/14/2009	0.65	9008	2917	0.821	17056	4899	0.566
8/16/2009	0.16	1821	37	0.113	3405	207	0.028
8/21/2009	1.45	20876	10801	1.897	39344	13078	1.812
8/22/2009	0.34	4462	1535	0.680	8419	2492	0.821
8/28/2009	0.87	12235	3744	0.821	23185	7148	0.991
8/31/2009	0.41	5488	1133	0.340	10369	2996	0.425
9/2/2009	0.02	31	0	0.000	31	0	0.000
9/7/2009	0.74	10329	1677	0.680	19563	5551	0.510
9/22/2009	2.74	40135	19246	1.558	75283	27340	1.359
9/25/2009	2.04	29684	16454	3.144	55781	16312	6.344
9/26/2009	1.3	18637	14831	1.473	35165	13633	1.416
10/5/2009	0.61	8422	770	0.255	15941	2031	0.227
10/10/2009	0.21	2555	507	0.368	4798	544	0.453
10/12/2009	0.15	1675	0	0.000	3126	34	0.011
10/14/2009	0.48	6515	1073	0.255	12320	1880	0.368
10/15/2009	0.05	208	0	0.000	340	3	0.001
10/24/2009	0.05	208	15	0.113	340	3	0.028
10/26/2009	0.31	4022	779	0.368	7584	685	0.425
10/28/2009	0.06	355	0	0.000	619	0	0.000
11/10/2009	0.26	3288	179	0.283	6191	960	0.453
11/11/2009	3.35	49242	30324	1.444	92277	45030	1.359
11/13/2009	0.15	1675	105	0.042	3126	731	0.142
11/23/2009	0.29	3728	193	0.142	7026	496	0.142
11/25/2009	0.06	355	0	0.000	619	0	0.003
11/30/2009	0.04	61	0	0.000	61	0	0.000
12/2/2009	2.6	38045	26312	1.954	71382	28402	1.756

I. Appendix: *Example* SAS code

I.1 Spearman Correlation Analysis (Chapter 2)

```
proc corr spearman data=sasuser.FF_correlate;
   with Log_Ecoli_EMC   Log_Fecal_EMC     Log_Entero_EMC;
   var Log_Ecoli_EMC Log_Fecal_EMC Log_Entero_EMC
   TSS_EMC TKN
      flow_duration volume peak_flow ave_flow
   rain_total storm_duration antecedent_dry Antecedent_since2
   Ave_intensity Max_5_min_intensity
   rain_time1 AT_time1 RH_time1 VP_time1 SR_time1
   rain_time2 AT_time2 RH_time2 VP_time2 SR_time2
   rain_time7 at_time7 rh_time7 vp_time7 sr_time7
   rain_time14 at_time14 rh_time14 vp_time14 sr_time14
   rain_time28 at_time28 rh_time28 vp_time28 sr_time28
   PET1 PET2 PET7 PET14 PET28;
   run;
   quit;
```

I.2 Multiple Linear Regression (Chapter 2)

```
proc reg data=sasuser.FF_correlate;
   *with Log_Ecoli_EMC ;
   model Log_Ecoli_EMC = flow_duration volume peak_flow ave_flow
   rain_total storm_duration antecedent_dry Antecedent_since2
   Ave_intensity Max_5_min_intensity
   rain_time1 AT_time1 RH_time1 VP_time1 SR_time1
   rain_time2 AT_time2 RH_time2 VP_time2 SR_time2
   rain_time7 at_time7 rh_time7 vp_time7 sr_time7
   rain_time14 at_time14 rh_time14 vp_time14 sr_time14
   rain_time28 at_time28 rh_time28 vp_time28 sr_time28
   PET1 PET2 PET7 PET14 PET28/selection=stepwise;
run;
quit;
```

I.3 Kruskal-Wallis (Chapter 2 - Code becomes Wilcoxon Rank Sum if only two seasons are compared)

```
data one;
input MPN season $;
cards;
4.511657096 Fall
4.218513404 Fall
4.096612728 Fall
3.540987783 Fall
4.039137799 Fall
3.667714754 Winter
3.950001498 Winter
4.129705753 Winter
2.851330389 Winter
3.944778099 Winter
4.109501129 Spring
4.664239537 Spring
4.927823966 Spring
4.643103574 Spring
4.773069453 Spring
4.429466115 Summer
3.651976234 Summer
4.873076    Summer
4.463602115 Summer
4.261970848 Summer


;
proc print;
proc npar1way wilcoxon data = one;
var MPN; class season;
run;
```

I.4 Wilcoxon Signed Rank (with t-test and Kolomgorov-Smirnov analysis)

```
data one;
input inflow outflow;
cards;
591   216
75
983
328   20
552   199
638   644
119   52
30    31
197   71
225   74
4839
75    3973
99    153
4210  3106
247   1034
922   372
2382  3466
1174  211
582   52
66    76


;


data b; set one;
ods select BasicMeasures TestsForLocation GoodnessOfFit;
ScoreChange=inflow-outflow;
proc univariate data=b;
var ScoreChange;
histogram/ normal;
run;
```